JOURNAL OF GENETICS

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, MANAGER
LONDON: FETTER LANE, E.C. 4

LONDON: H. K. LEWIS AND CO., LTD., 136 Gower Street, W.C. 1
LONDON: WILLIAM WESLEY AND SON, 28 Essex Street, Strand, W.C. 2
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(Agent for the United States and Canada)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN AND CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

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JOURNAL OF GENETICS

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Volume X. 1920

168870.
24/1/22.

Cambridge
at the University Press
1920

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431
A1J64
v.10
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ERRATA

Owing to an error in alignment the Table on p. 314 of Vol. IX. is given incorrectly. It should read as follows :

TABLE II.

Grades	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Calculated Distribution	—	5	10	10	10	5	10	5	5	—	—	—	—	5	15	15	17	7
Actual distribution from Castle	—	2	3	4	11	11	14	9	3	1	—	—	—	1	14	24	13	6

VOLUME X JULY, 1920 No. 1

A PRELIMINARY NOTE ON THE FLOWER COLOUR AND ASSOCIATED CHARACTERS OF THE OPIUM POPPY.

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Assistant to the Economic Botanist.

(With Plate I.)

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INTRODUCTION.

PREVIOUS to the outbreak of war in 1914 the crude opium used for medicinal purposes was mainly derived from the Turkish markets which, in their turn, were dependent on the cultivation of the Opium Poppy in Asia Minor and the Balkans. On the entry of Turkey into hostilities with the Allies this source of supply was cut off, and attention was

directed to India as a possible field from which the demand could be met. Indian Opium had, in the past, failed to compete with the Turkish product mainly because the morphine content was, as a rule, below that required by the B. P. With Turkish competition removed, the trade was forced to accept Opium of lower morphine content than that to which it had been accustomed; that acceptance was, however, not willingly given and the enhanced price paid for Opium satisfying B. P. requirements raised the question as to the possibility of producing, in India, an Opium of high morphine content. The desirability of affecting such an increase of the morphine content is of more than immediate importance. The medical Opium trade is, in value, in the neighbourhood of £1,000,000 per annum and has hitherto been in foreign hands. The present time, consequently, offers a great opportunity of replacing permanently the foreign product by one produced within the Empire. With this object in view the question was referred to us by Government and has formed the subject of experiments which are still in progress.

The Opium Poppy grown in India is distinct from that grown in Turkey and appears to have received little attention botanically. Clearly such an investigation, with a view of isolating as many races as possible which could later form the basis for chemical investigation, was the first thing desired. For this purpose large numbers of samples of seed were collected, and from these pure races isolated. While the Opium Poppy of the main opium tract, situated in the United Provinces, is white flowered, that of the Malwa tract contains coloured races and the range of colour is considerable. This petal colour forms one of the most prominent characteristics of the plant and has, therefore, been used as a primary character in determining the purity of the races having coloured flowers, and the amount of work done on these has been considerable. The economic problem, as described above, is, however, fundamentally chemical, and representation of this aspect led to the association of Mr Annett with us to investigate the chemical side of the problem. The investigations hitherto carried out indicate that the solution of the economic problem, to which circumstances compel attention to be restricted for the present, is not to be found in any of the coloured races. Were no other characters to be considered, the branching habit so characteristic of the coloured races, leading to the production of as many as twenty-four or even thirty heads on one plant, makes these unsuitable for cultivation with the object of drug production, for the method of collecting the drug, by lancing the capsule at a certain stage of maturity, makes the production of a single capsule

only per plant one of the most desirable features, since the maturation of the crop will thus be confined within the narrowest limits of time. Such a single capsuled habit is typical of most of the white flowered races of the United Provinces. Chemical investigation has, further, shown that some of these races yield opium containing as much as 18 °/₀ morphine, and there is the possibility that these coloured races might afford material for breeding a high morphine content on to the habit of the United Provinces races, thus producing a combination hitherto nonexistent. The chemical work has, moreover, indicated a simple and efficacious method by means of which opium of between 10 and 14 °/₀ morphine content can be procured in any quantity from the races as at present grown. While, therefore, there is considerable scope for improvement by breeding within the United Provinces races, there appears to be small prospect of the coloured races affording any economic material in the solution of the immediate problem.

The object of the present paper, however, is not the development of the economic aspect. The work on the coloured series, involving as it does a record of over 150,000 plants in the season 1918 alone, has yielded results which appear worthy of record. These are, however, of a preliminary nature only, and would not have been published but that the necessity for concentrating on the economic aspects renders it doubtful if time will be found for further investigation.

Literature.

No complete examination of the Opium Poppy appears to have been made from the breeder's aspect. From the agricultural aspect a certain amount of work has been undertaken, but the plant is here considered mainly as an oil producer, and its drug yielding capacity has been ignored.

A series of investigations are recorded by Hoffman[1] between 1872 and 1884 in which reference is made to flower and seed characters of the Opium Poppy among several other species of the Papaveraceae. De Vries[2] has similarly recorded observations on the Opium Poppy and the results of certain crosses in which the same characters figure, while Fruwirth has summed up the results of his own experiments[3] in "Die Zuchtung der landwirtschaftlichen Kulturpflanzen," where a full list of

[1] See especially *Bot. Zeit.* L. (1882), 499.

[2] *Die Mutationstheorie.*

[3] *Naturw. Z. f. L. u. F.* II. 1904, 1.

references to previous work is given. Since that date no extensive work appears to have been undertaken, and the plant to which reference is made throughout is the *P. somniferum* L. of the garden, or of the Continental poppy field where cultivation is undertaken for seed.

The Indian Opium Poppy differs in certain respects from the above. The season in which it develops is a short one and it has so far been found impossible to synchronise the flowering period of the imported races with those of the Indian. On the true Indian races practically no work has been done since Scott[1], in 1877, described those recognised by the cultivator. The plant was selected by the Howards and Abdur Rahman[2] for the purpose of gaining some information as to the extent to which cross-fertilisation occurred in the commoner Indian crops, but no attempt was made to identify the factors concerned.

The Petal Colours of the Opium Poppy.

As has been stated, the poppy of the main opium producing tracts of the United Provinces is white flowered. Stray plants with red petals occur and also a form with the petal sepaloid (called small petal, *s*, in this paper). The majority of the coloured forms are derived from the poppy grown in Malwa and the range of colour in these is considerable[3]. In spite of this apparent complexity, however, the series may be arranged in five groups, which are determined by the several combinations of the following four factors:

P, a factor which develops a pink marginal colour in the petal leaving the eye colourless. In the pure form the colour is strongly developed (Pl. I, fig. 1), while in the form impure for this factor the colour is light but distinct (*LP*), or so pale (*VL*) that a minute examination is required to avoid confusion with the white (*W*). Typically the colour is pure pink, but in certain cases it may appear distinctly crimson by transmitted light.

R, an intensifier of P. In the absence of the latter the presence of R cannot be determined but, in conjunction with P in the pure form, it develops a form (*RP*) with a scarlet or crimson marginal colour which is separated from the white eye by a blue or purple band (Pl. I, fig. 3).

[1] J. Scott, *Manual of Opium husbandry*, 1877.

[2] *Memoirs Dept. of Agr. in India*, Bot. Series, III. 10, 321.

[3] Several extra-Indian forms have been grown in addition. These include the true Turkish form, seed of which was kindly supplied by Mr L. Sutton. These forms are not dealt with in the present paper.

This band may be narrow and sharply defined, broad and diffuse. Associated with *P* in the impure condition, it develops a form (*PW*) having a pale purple margin and white eye (Pl. I, fig. 2). In certain families the *PW* form approximates so closely to the crimson *RP* form that correct determination on appearance is hardly possible.

M, a factor which develops a mauve-purple in the margin and a deeper eye (Pl. I, fig. 4). As in the previous cases, this, again, may be divided into two forms, a standard form, and one showing a distinct crimson form by transmitted light. This factor shows complete dominance, and the purity or reverse of any particular plant for this factor is, consequently, only determinable by further culture.

L. The nature of this factor forms the subject of discussion later; for the present it may be considered an intensifier of the factor **M**, converting the mauve-purple into a rich magenta-purple (Pl. I, fig. 5) with an associated form in which a crimson shade is apparent by transmitted light. The degree of intensification is, however, very variable and, in practice, the entire purple group forms a continuous series in which, while it is possible to distinguish with certainty plants which carry the factor **L**, it is not possible to distinguish those lacking this factor with the same degree of certainty. It is impossible, thus, to separate a mixed culture into two groups according as they do, or do not, possess that factor.

The five groups which arise from the combination of these factors are as follows:

I. *The white-eyed group.* In this group the **P** and **R** factors occur, but the **M** factor is absent. The following forms are recognisable:

Pprr	*VL* or *LP*	very light, or light pink
PPrr	*P*	full pink
PpRr / **PpRR**	*PW*	purple white eye
PPRr / **PPRR**	*RP*	red-purple white eye

II. *The purple group.* In this the **M** factor, or the **M** and **L** factors, occur, but from it the **P** factor is absent.

The various forms are all purple. When the **L** factor is absent the colour is invariably a light mauve-purple, *MP*, but when it is present, a series, varying from the true mauve-purple to a full magenta-purple, *PL*, is obtained.

III. *The dilute colour group.* In this group the marginal colour is dilute and the eye coloured. Both the **P** and the **M** factors are present,

but the L factor is absent. The R factor, if present, has no visible effect in presence of the factor M. The following forms are distinguishable:—

PPMM / PPMm — *PP* — Pink-purple eye (Pl. I, fig. 6). The marginal colour is pink but the eye is coloured.

RB — Red-blue eye (Pl. I, fig. 7). The marginal colour, judged on appearance only, would be termed full. The experiments detailed below, however, show that it is in reality a dilute colour.

PpMM / PpMm — *PPC* — Judged by appearance this would be termed the crimson form of *PP*, bearing the relation to that colour that the crimson forms of *P* and *RP* bear to the standard forms of those two colours. It is, however, a relation which is not borne out by experiment.

PP_2C / *PP_2P* — Two dilute forms which are, in the typical condition, distinct. Intermediate forms, however, occur and a practically continuous series is thus formed, the end term of which approximates to the *MP* form. The best idea of these forms is, perhaps, given by supposing a blue anthocyanin colour to be superimposed on the pink of the *PP* form, the eye at the same time becoming diffuse. (Pl. I, fig. 8.)

IV. *The full colour group.* The marginal colour is in all forms full and the eye coloured. Factorially considered it is in all respects similar to the last (III) group but the factor L is also present. The following forms are recognisable:

PPMMLL (or Ll) / PPMmLL (or Ll) — *R* — Red (Pl. I, fig. 9). This colour is familiar as that of the wild poppy of the English corn fields, the eye being typically small and deep purple, sharply defined. A crimson form occurs in which the marginal colour is distinctly crimson by transmitted light. This series may be divided into three sections according to the size of the eye R_1, R_2, R_3, and each section would appear to possess a corresponding crimson form C_1, C_2, C_3. When, however, the eye attains its maximum development the marginal area is so reduced that the presence of a crimson form cannot be determined with certainty.

PpMMLL (or Ll) / PpMmLL (or Ll) — *CPC* — Crimson-purple with a crimson tendency (Pl. I, fig. 10). This is clearly not a pure colour, a blue anthocyanin being superimposed on the red or crimson marginal colour. In extreme cases it may be confused with C_2 above.

CPP — Crimson-purple with a purple tendency. This form is similar to the last but the superimposed colour is sufficiently deep to mask the red. The marginal colour is, thus, very deep and approximates in cases to the deepest *PL* form.

V. *A white-flowered group.* In this group the factors P and M are absent and the entire petal is pure white.

One further colour must be mentioned, namely that to which the term *F_1C* has been applied. In appearance it forms a connecting link between the *PP_2C* and *CPC* forms, the eye being distinctly bluish and diffuse. As its name implies, this form was first identified in the F_1 generation of certain crosses, in some of which it appears to represent *PP_2C* forms; while, in others, the *CPC*. The reason why certain artificial crosses should develop a colour which is not recognised in the natural mixtures is, as yet, not apparent.

The P *factor.*

Parents possessing a full pink colour in the petal invariably breed true and are therefore pure for the factor, or factors, developing that marginal colour.

The light pink, *VL* and *LP*, forms are impure, and the figures which follow indicate that a single factor only is concerned. To this factor we have given the designation P, and the *VL* and *LP* forms, therefore, have the constitution Pp.

Number of families	*W*	*LP* or *VL*	*P*
63	3671	8284	4480
1 : 2 : 1 expectation	*4109*	*8218*	*4109*

Crosses *W* by *P*. F_1		all *LP* or *VL*	
4 F_2	9240		2891
3 : 1 expectation	*9099*		*3033*

In the case of three of the crosses the F_1 and the impure F_2 plants were of the form *VL*, which, as has been stated, is with difficulty distinguished from the pure white. For this reason the count has, in these cases, been made jointly to include both forms.

The figures given for these 63 families illustrate a feature which, as it is one which will have to be borne in mind in considering all the results which follow, we may refer to here, though the matter will be dealt with later in more detail. Stated briefly, it is the existence of a correlation between the presence of the factor which develops the pink marginal colour and the length of the vegetative period of the plant. When the factor, which we have termed P, is present, a marked shortening of the vegetative period occurs and this phenomenon introduces certain practical difficulties which cannot be disposed of altogether.

In agreement with the common practice the seed is sown on land which has been irrigated and ploughed, and germination consequently takes place on a rapidly decreasing moisture. Under the conditions of experiment, a variable proportion of the seed will fail to germinate on sowing, but will do so on the first irrigation of the growing crop. Such plants constitute a second growth, of which only the more rapidly maturing plants will flower before the dry winds of the early hot weather kill all plants still in the vegetative condition. This pre-flowering death-rate is consequently greatest in those late maturing plants which do not carry the factor P, and counts thus show a deficiency in the number of such plants. This source of error cannot be

entirely eliminated, though care in sowing to bring the seed into intimate contact with the soil and the elimination of the second crop after germination will do much, but neither of these methods is entirely practicable in cultures which extend over many acres. The point is well illustrated in the above table which, in the case of the 63 families, shows a very definite deficiency in the number of whites, and a corresponding excess in the number of pinks. The absence of that discrepancy in the case of the crosses is also explainable. These crosses were grown on light soil, the friable nature of which permitted an intimate contact between the seed and the soil particles and in it, also, the movement of soil moisture is well maintained. This latter factor prevents the too rapid drying out of the surface soil in which the seed lies, and the moisture available for germination thus persists for a longer period in such soils than in the heavier soils on which the 63 families were grown. The F_2 crop was, thus, almost completely free from the intermixture of a second crop, and for this reason the deficiency in the number of whites is not apparent.

The M *factor.*

It has been stated above that the purple flowered plants which are denoted by *MP* owe their colour to a single factor M, but that this colour is merely the end term of a series of which the other extreme is the *PL* form. This latter colour is only developed when an additional factor, L, of the nature of an intensifier, is also present. Without collateral evidence as to the true constitution of the purple flowered plant it is not possible, therefore, to say with certainty in all cases whether the factor L is present or not.

The number of purple flowered plants which have been shown to give only purple flowered offspring amounts to several hundreds, and there is no question, therefore, that the purples can exist in a condition of purity. The behaviour of the impure purples is given below.

Number of families	Purple	White	
6	1855	564	Collateral evidence proves absence of L
20	9678	3168	Collateral evidence proves presence of L in condition Ll
28	15206	4704	Collateral evidence proves presence of L in condition Ll
77	13015	4052	Collateral evidence wanting
Totals 131	39754	12488	
Expectation 3 : 1	*39182*	*13060*	

These figures indicate sufficiently that the development of the purple colour is due to a single factor, which we have termed **M**; and this conclusion is confirmed by the series of crosses which have been made between *MP* or *PL* and white (*W*) and their reciprocals.

Number of families	Purple	White
4	11963	4250
Expectation 3 : 1	*12160*	*4053*

The R *factor.*

The nature of this factor as an intensifier, changing the *P* into the *RP* form, is made clear in the following table:

	Number of families	*W*	*LP*	*P*	*PW*	*RP*
PPRr	19	—	—	1411	—	3978
	Expectation 1 : 3	—	—	*1347*	—	*4041*
PpRR	117	6469	—	—	15539	7763
	Expectation 1 : 2 : 1	*7443*	—	—	*14886*	*7443*
PpRr	32	2309	1039	594	3829	1919
	Expectation 4 : 2 : 1 : 6 : 3	*2424*	*1212*	*606*	*3636*	*1818*

The approximation between the actual figures and those demanded by our assumption is sufficiently close when due allowance is made, for the reasons already given, for the deficiency in the number of whites.

The conclusion is confirmed by the results obtained from the crosses which have been made.

	W	*LP*	*P*	*PW*	*RP*
W by *P* and reciprocal F_1 all *PW*					
F_2	962	—	140	755	401
Expectation 6 : 1 : 6 : 3	*846*	—	*141*	*846*	*423*
W by *RP* and reciprocal F_1 all *PW*					
F_2	1229	—	—	2394	1247
Expectation 1 : 2 : 1	*1217*	—	—	*2436*	*1217*

In both cases the expectation is based on the assumption that the white parent carries the factor **R**.

Further proof, were it needed, has been furnished by crossing two plants each of some 372 apparently pure white races, which were under single plant culture, by a pure pink. Of these, 82 have given an *LP* or *VL* F_1, indicating that the white parent lacks the **R** factor; 279 have given a *PW* F_1, and of these the white parent is probably pure for the **R** factor, while 11 have given a mixture of *LP* and *PW* plants indicating parental impurity for the **R** factor.

Effects of P *and* M *in absence of* L.

The following table collates the results of all those families in which the L factor is not present:

Number of families	*W*	*LP*	*P*	*PW*	*RP*	*RB* *PP*	*PPC* *PP_2C* *PP_2P*	*MP*
Parent *RB* or *PP* (PPMM) all breed true Parent *RB* or *PP* (PPMm)								
26	—	—	1070	—	—	3718	—	—
Expectation 1 : 3	—	—	*1197*	—	—	*3591*	—	—
Parent *PPC* or PP_2 (PpMM)								
192	—	—	—	—	—	13875	26693	12158
Expectation 1 : 2 : 1	—	—	—	—	—	*13181*	*26364*	*13181*
Parent *PPC* or PP_2 (PpRRMm)								
29	263	—	—	603	306	946	1907	874
Expectation	*306*	—	—	*612*	*306*	*918*	*1836*	*918*
Parent *PPC* or PP_2 (PprrMm)								
27	381	827	448	—	—	1289	2504	1091
Expectation	*409*	*818*	*409*	—	—	*1226*	*2452*	*1226*
Parent *PPC* or PP_2 (PpRrMm)								
23	186	157	98	323	221	798	1266	568
Expectation	*215*	*163*	*56*	*338*	*169*	*677*	*1354*	*677*

Not only do the combinations expected on the two factor basis all appear but, when due allowance is made for the deficiency in the number of whites and purples, which both lack the factor P, and are therefore late flowering, the numerical results point to the same conclusion.

The above table also indicates that the factor R is in no way correlated with either of the two factors P or M, a fact which requires to be established in view of subsequent discussion.

The same conclusion is arrived at when the crosses are considered.

		W	*LP*	*P*	*PW*	*RP*	*RB*	*PPC*, PP_2	*MP*
RB by *MP*	F_1 all F_1C								
	F_2	—	—	—	—	—	490	1006	592
MP by *P* and reciprocal	F_1 all PP_2								
	F_2	277	473	267	—	—	843	1705	789
	Expectation	*272*	*544*	*272*	—	—	*816*	*1633*	*816*
MP by *RP* and reciprocal	F_1 all PP_2								
	F_2	229	103	87	374	180	809	1511	655
	Expectation	*246*	*123*	*62*	*370*	*185*	*740*	*1481*	*740*

The two latter crosses thus constitute a direct synthesis of the *RB* form from the *P* and *MP* forms.

Effects of P *and* M *in presence of* L.

For simplification of tabulation the white-eyed forms are here grouped together. The full records, however, show that the expected forms are represented and in due proportion, whether as *LP* and *P* only, when the R factor is absent; as *PW* and *RP* only, when the R factor is present in the condition of purity, or as all four forms when the R factor is present but in the impure condition.

(*a*) *The* L *factor pure,* LL.

If we assume the L factor to be merely an intensifier, its presence in the pure condition will be indicated by a series corresponding to the last but by a series in which the *RB* and *PP* forms are replaced by an *R* (R_1'', R_2, or R_3), and the *PPC* and PP_2 forms by the *CPC* or *CPP* form.

Number of families	*W*	*P*	*RP*	*R*	*CPC″, CP*	*PPL*
Parents red (PPMM) all pure red						
Parents red (PPMm)						
21	—	1255	—	4204	—	—
Expectation	—	*1365*	—	*4094*	—	—
Parents red (PPRRMm)						
9	—	—	922	3071	—	—
Expectation	—	—	*998*	*2995*	—	—

Number of families	*W*	White eyed forms	*R*	*CPC, CPP*	*PL*
Parent *CPC* or *CPP* (PpMM)					
290	—	—	31306	59835	20604
Expectation	—	—	*27936*	*55873*	*27936*
Parent *CPC* or *CPP* (PpMm)					
55	749	2972	3644	6560	2584
Expectation	*1033*	*3099*	*3099*	*6199*	*3099*

The deficiency in the number of whites and purples is large and is associated with an excess in the number of reds. There is here, also, a further complication which arises from the confusion which we have shown to arise in certain cases between the C_2 and *CPC* forms (see under the description of the *CPC* form above). If we take only those families in which such confusion cannot arise, those, that is, in which the PPMM form is R_1 or C_1, we obtain the following figures:

Number of families	*R*	*CPC*	*PL*
31	9201	18571	5259

In these the ratio 1 : 2 is very nearly attained between the *R* and *CPC* forms, while the deficiency in the number of purples remains very marked.

The figures yielded by the crosses are in similar accord with the explanation offered.

	W	*LP* and *P*	*RP* and *PW*	*R*	*CPC, CPP*	*PL*
Parent *R* by *PL* or reciprocal,		F_1 all *CPC*				
F_2	—	—	—	3445	6860	3178
Parent *R* by *W* or reciprocal,		F_1 all F_1C				
F_2	941	2504	—	2578	5496	2587
Parent *R* by *W* or reciprocal,		F_1 all F_1C				
F_2	322	—	923	1194	2800	1532
Parent *R* by *W*,		F_1 all F_1C				
F_2	133	133	286	460	1010	475
Parents *PL* by *P*, *PL* by *RP* or reciprocals,		F_1 all *CPC*				
F_2	128	106	228	356	741	352

Here again the deficiency in the number of whites and purples is less marked and confirms our previous conclusion that the effect is traceable to the soil in which the F_2 crop was grown.

In the latter case we have an example of the direct synthesis of the *R* form.

(*b*) *The* L *factor impure,* Ll.

The evidence derived from families in which the L factor appears to be impure is contained in the following table:

Number of families	*W*	Group II colours	*RB*	*PPC, PP*$_2$	*R*	*CPC, CPP*	*PL'', MP*
Parent *R* (PPMMLl)							
1	—	—	92	—	151	—	—
Parent *CPC* (PpMMLl)							
29	—	—	615	1118	1790	3554	1949
Parent *CPC* (PpMmLl)							
20	245	1085	305	557	853	1586	910
F_2 crosses							
6	349	1137	363	543	674	1535	879
Totals ...	594	2222	668	1100	1527	3121	1789
Expectation	*687*	*2063*	*516*	*1032*	*1547*	*3094*	*2063*

The recorded results are thus in fair agreement with expectation based on the assumption that the L factor is of the nature of an intensifier, the main point of divergence being the large excess of *RB* forms.

The white-flowered group.

It would appear from the above results that the white form, in spite of its apparent uniformity in petal colour, is in reality a diverse group in which at least two independent factors of the nature of intensifiers may exist. The evidence which we have given in the above tables proves that the **R** and **L** factors are not one and the same. The presence of the factor **R** in some and not in other whites has already been given, and the same argument applied to the crosses tabulated above (p. 12) may be used to prove the presence of the **L** factor in certain white (as well as pink and *RP*) parents.

Of greater interest, however, are those cases in which an extracted white has been obtained from the cross between two pure coloured parents. It would seem to indicate the presence in the poppy of two anthocyanin bases as capable of coexistence as of independent existence, and each capable of developing independently a colouring matter. The phenomenon is in marked contrast to the more commonly known instances in which the purple, or blue, colour is derived from a pink or red and is, consequently, not capable of independent development.

The **L** *factor.*

We have, throughout the above discussion, assumed that the **L** factor is of the nature of an intensifier, and all the results so far detailed are concordant with that supposition. The matter cannot be so readily dismissed, however. In the crosses (p. 10) the *RB* form was synthesised by crossing the purple, *MP*, form by the pink, *P*, and by the *RP*, forms. It follows from this synthesis that, if the **L** factor is an intensifier, these *P* and *RP* parents cannot carry that factor. These parents should, therefore, when crossed with a purple of the form **MMLL**, give an F_1 having the constitution **PpMmLl**, yielding among others *RB* and *PPC* forms in the F_2. Actually this is not so, for the same parents occur among the families recorded on p. 10, in which the dilute group does not occur. Again, an *MP* parent which, when crossed with a *P*, has given no Group IV colours in the F_2, has, when crossed with an *R* form, failed to give any Group III colours. The former result indicates that the *MP* plant has the constitution **MMll**, the latter that the constitution is **MMLL**.

The first of these two cases, if considered independently, would support the conclusion that the **M** and **L** factors are independent, and

both capable of developing a purple colour; but such a conclusion is not supported by any other evidence. If, for instance, we adopt this supposition, of the extracted purples from those families in which both Group III and Group IV colours occur, 7 should be pure, 4 should give purples and whites in the ratio 3 : 1, and 4 the same colours in the ratio 15 : 1. Actually, of the purples bred on for another generation from these families, 13 have proved to be pure, 20 have given purples and whites in the proportion 3 : 1, while none have given the ratio 15 : 1. Nor have we observed in any of the numerous families raised from impure purples a single instance of a 15 : 1 ratio.

A third explanation, namely that the two factors **M** and **L** are alternative, occupying identical loci in the chromosome[1], is also ruled out of the results we have recorded. The form *PPML*, which alone carries the two factors on this supposition, could not give Group II and V colours.

For the present we are unable to offer any completely satisfactory explanation of the phenomenon here described, and further work will be necessary before such an one is obtained. We have already referred to the uncertainty which attaches to the form F_1C. A more complete understanding of the nature of this colour may afford the explanation at present lacking.

Other colour factors.

In describing the colour groups we noted that, in most cases of the pure colours, each form is in reality dual. On the one hand is the standard form and on the other that which develops a distinct crimson appearance especially marked when the petal is viewed by transmitted light. Thus the pink occurs as the standard, *P*, and the crimson-pink *P* (*C*); the *RP* as the standard and the crimson *CP*; the *MP* and *PL* as standard and as the crimson *MPC* and *PLC*; the *RB* as the standard and the crimson *CB* and, finally, the red as the standard *R* and the crimson *C*. In certain families the standard, and in others the crimson form occurs in a condition of purity. In other cultures, again, a complete series ranging from the typical standard to the typical crimson is obtained. It would appear that the crimson colour is due to a factor of the nature of an intensifier which is activated both by the **P** and **M** factors. It is possible that two factors are involved, but the evidence is as yet insufficient for a definite decision to be given on this point.

[1] See Morgan, *The Mechanism of Mendelian Heredity*, Chap. VII.

The distinction between the two forms, *RB* and *PP*, of plants which bear apparently the same constitution remains unexplainable on this basis for the typical *CB* variation of *RB* has been shown to occur. The apparently similar difference between *PP* and *PPC* is, however, a phenomenon of a different order for, as we have shown, the latter form is impure for the factor P.

In describing the red form we noted that there appeared to exist at least three distinct sub-forms which we denoted by R_1, R_2, R_3, and that these differed in the size of the eye. These differences, again, appear to be due to a factor or, more probably, a group of factors affecting the size of the eye. The same feature is observable in the Group I colours. In the pink forms, for instance, the colour may be so restricted that, when the petal is laciniated, it is impossible to say definitely on observation alone whether the plant is of the *P* or the *LP* form.

Certain other observed differences in flower colour appear to depend on this group of factors. Thus the impure form *PPC* is associated with the pure *PP* form, while the impure forms PP_2C and PP_2P are associated with the pure *RB*. This association indicates that the difference between the *PP* and *RB* forms must be sought in this group of factors. Again, the observed difference between the two impure forms *CPC* and *CPP* appear to depend on the same group of factors, for the former is associated with the R_1 pure form, while the latter is associated with the R_2 and R_3 pure forms.

The Shape of the Petal.

In the majority of the United Provinces' races the petal is entire. In certain forms only is the margin of the petal sected, that is, divided by a few cuts extending to a depth of about half an inch. This sected petal is associated with the particular form of leaf found in what are known as the Katela races to the extent that, while all the Katela races have the sected petal, certain forms have the sected petal with the common form of leaf. The character is very indefinite, the extent to which secting occurs varying from a single cut on one petal to eight or ten cuts on each of the four petals. The character has not formed the subject of experiment.

In many of the Malwa races the margin of the petal is deeply cut and the petal is laciniated (Pl. I, fig. 11). This character, in as much as it divides the margin into a large number of distinct segments, increases the difficulty in determining the marginal colour.

In addition to the above there occurs, at rare intervals, a form in which the petal is much reduced in size. In the extreme case the petal is sepaloid and the flower fails to open. Intermediate forms occur in which the petals, though much reduced in size, open and have a distinct petaloid appearance. This group of plants is referred to as the small petalled forms (*s*) (Pl. I, fig. 12).

The laciniated petal.

In numerous cultures the laciniated petal has occurred in an impure condition and in a few of these counts have been made. Counts have also been made in a few cases when one parent of a cross has possessed the laciniated petal while the petal of the other parent was entire.

	Number of families	Laciniated	Entire
	11	3471	1443
Crosses	3	6565	1553
Totals ...		10036	2996
Ratio		3·36	1

From these figures it is probable that the laciniation is due to a single factor showing complete dominance.

The size of the petal.

The ratio of small petalled plants to those bearing the normal petal is, in the case of families impure for the character, 1 : 4·5 on a total of over 45,000 plants and, in the case of the crosses, 1 : 3·9 on a total of over 2,800 plants. These figures, considering the number of plants recorded, indicate a very considerable excess of plants with the normal petal—an excess which, occurring as it does with considerable regularity in each family, appears to be too great to be fortuitous.

The factor appears to be a simple one, however, and associated in no way with the colour factors. Consequently, corresponding to each colour form, there will occur an *s* form bearing the particular colour factor, or factors, which develop that colour. Owing, however, to the reduced size of the petal these forms are not fully capable of identification by appearance. The white form is represented by a form having a greenish colour (Pl. I, fig. 12 *b*), while those corresponding to the Group I colours are recognisable as distinct from other forms but are not distinguishable *inter se* (Pl. I, fig. 12 *a*); that corresponding to the purple, especially the *MP*, form is frequently so lightly coloured as to be hardly separable from the white, while the forms corresponding to the colours

of Groups III and IV build a series which cannot be separated into its components (Pl. I, fig. 12 *c*). The true ultimate colour constitution can, thus, only be determined by breeding.

The Seed Colour.

The colour of the seed is derived from a pigment of the testa and is, therefore, a parental character. The following colours occur:

1. *White.*
2. *Straw.*

The above two colours have not been distinguished in any breeding experiments, and may be considered for the present to constitute a single group—the white seeded group.

3. *Pink.*

This colour covers two forms, of which the seed coat appears respectively opaque and transparent. Both forms occur in the same capsule, and the cause of the difference, whether dependent on maturity or a racial character, has not been worked out. In the few observations on seed colour the pink is considered a simple character.

4. *Blue.*

Here again there are two forms, the one light, the other deep, blue and both may occur in the same capsule. Races with pure light, and with pure dark, blue seed have been isolated. As in the last case, however, the blue character is treated as a simple one.

5. *Brown.*

This colour has only been found pure in a single culture from a single plant, and in a few stray plants. For the present, beyond noting the existence of the form, nothing can be recorded about it, and the character forms the basis of a series of experiments as yet incomplete.

The first striking feature connected with the seed colour is that the division between the group with colourless, and that with coloured seed coincides with the division that can be drawn by the eye of the petal; Groups I and V, with the eye colourless, being white seeded, while Groups II, III and IV, in which the eye is coloured, invariably have coloured seed. Among the hundreds of thousands of plants which have passed under observation no single exception has been observed. It follows from this that the ratio of coloured to colourless seeded plants is in all cases 3 : 1.

The pink colour.

Where the culture contains Group II, III and IV colours only, whether singly or in combination, the plants with pink seed breed pure for that character. If the parent flower has been protected the purity is complete; in those cases, however, where such protection is not given, a few plants with blue seeds may occur among the offspring, indicating a dominance of the blue colour in these natural crosses.

The blue colour.

Many of the cultures raised are pure with regard to this character, while others contain both blue and pink seeded plants in the proportion 3 : 1.

Number of families	Blue	Pink
5	368	124
Expectation 3 : 1	*369*	*123*

It follows from this that the blue testa colour is developed from the pink by the action of one factor only. Where the offspring contain plants having the Group I and V petal colours also, the ratio 9 Blue : 3 Pink : 4 White is necessarily obtained from which it follows that a white seeded form which carries the factor for blueness must exist. Confirmation of this conclusion by the synthesis of a blue seeded plant from a cross between white and pink seeded parents has yet to be effected.

The Correlation between the Vegetative Period and the Flower Colour.

One case of complete correlation has already been dealt with, namely that between the seed and eye colours. A second, and very marked, one has already been referred to earlier in as far as it bears on the interpretation of the counts which have been given for the flower colours and will here be considered in greater detail. It is that correlation which has been found to exist between the length of the vegetative period and the flower colour. In the accompanying table are given a few characteristic examples of such correlation taken from the simpler cultures. From this table it follows that the presence of the factor which develops the *P* colour from white, of that which develops the *PP* or *RB* colour from purple (*MP*), and of that which develops the *R* (*C*) colour from purple (*PL*) is in each case associated with a reduction in the length of the vegetative period. While the observation gives no

proof that these three factors are one and the same, it does afford a certain amount of support to this conclusion; and it indicates that the difference between the Group III and IV colours is most probably to be sought in the factor developing the purple colour or in some factor associated therewith.

Correlation between vegetative period and the P *factor.*

Date	*W*	*LP*	*P*
January			
10/17	—	—	8
19	—	—	7
21	—	—	9
22	—	—	4
24	—	3	6
26	—	3	4
28	—	1	3
30	—	3	1
February			
1	—	8	1
3	—	8	1
5	—	3	—
7	—	9	—
9	—	10	—
11	3	18	—
13	2	13	—
15	2	3	—
17	16	5	—
19	3	4	—
21	9	3	—
23	6	2	—
25	7	—	—
27	7	—	—
March			
1	3	—	—
3	4	—	—
5	—	—	—
7	1	—	—

Date	*W*	*PW*	*RP*
January			
6/11	—	—	13
13	—	—	10
15	—	—	14
17	—	2	13
21	—	—	16
22	—	2	3
24	—	5	8
26	—	6	2
28	—	6	3
30	—	13	—
February			
1	—	21	1
3	—	13	1
5	—	19	2
7	—	11	2
9	1	16	1
11	3	18	—
13	8	11	—
15	7	13	—
17	2	13	—
19	6	14	—
21	9	6	—
23	7	3	—
25	6	3	—
27	7	—	—
March			
1	13	—	—
3	5	—	—
5	2	—	—

Date	*MP*	*PPC*	*PP*
January			
9	—	—	1
13	—	—	1
15	—	—	3
17	—	—	7
19	—	—	6
21	—	—	7
23	—	—	1
24	—	—	2
27	—	5	9
29	—	—	10
31	—	2	1
February			
2	—	13	8
4	—	13	1
6	1	10	1
8	1	15	1
10	2	15	—
11	2	5	—
13	2	10	—
16	4	5	—
18	2	3	—
19	5	1	—
21	5	2	—
23	3	1	—
25	5	1	—
27	3	—	—
March			
1	4	—	—
3	6	—	—

Date	*MP*	*CPC*	R_2
January			
10/16	—	—	19
18	—	—	14
20	—	—	17
21	—	—	10
23	—	—	18
25	—	—	14
27	—	—	15
29	—	—	5
31	—	3	9
February			
2	—	2	11
4	—	7	7
7	—	10	2
8	—	5	—
10	—	16	3
12	—	20	—
14	1	47	—
16	2	42	—
18	4	39	—
20	14	17	—
22	9	31	—
24	14	17	—
26	21	10	—
28	15	4	—
March			
2	6	—	—
4	12	—	—
6	2	—	—
8	5	—	—
10	2	—	—
12	2	—	—
14	1	—	—

A further point worthy of note is what may be termed the partial dominance of the P factor as indicated, not only by the flower colour, in which the heterozygous form P is distinct from the pure form, but by the intermediate length of the vegetative period of the impure form. The difference in the length of the vegetative period is sufficiently great to leave an interval of a week or more between the early flowering (PP) and the late flowering (pp) forms. During the interval only the intermediate, impure, flower colour will appear. Casual visits to the plots may, therefore, lead to very erroneous conclusions as to the character of

a culture which, according to the time of the visit, may appear pure to one or other of three forms, one of which is not, in reality, a pure form at all. Before this correlation was established a number of cultures were noted as being pure to the earlier form, and the counting discontinued a few days before the second, or intermediate, form first made its appearance. The table illustrates the simpler examples of this phenomenon, which is, however, equally well shown in the more complex cultures. For instance, a culture containing a complete mixture of Groups I to V will at first appear to be a mixture of *P*, *RP*, *RB*, *PP* and *R* forms; later, as these disappear, *VL*, *LP*, *PW*, *PPC*, PP_2, and *CPC* take their place, and ultimately these two are replaced by the *W*, *MP* and *PL* forms.

Before leaving this subject it may be noted as a fact, of which no explanation can be offered, that this correlation has failed to appear in the F_2 of the crosses which have been made, and which would be expected to show it. Such a total disappearance in the progeny of the crosses of a phenomenon, which is of universal occurrence in the single plant cultures, can only be regarded as extraordinary, requiring for explanation a more detailed study of the history of the parents than has yet been undertaken.

This preliminary review has indicated one or two points which are not without interest, and which, now that the war is over, will, it is hoped, form the subject of further investigation.

EXPLANATION OF PLATE I.

Fig. 1. Pink, white-eyed (*P*).
Fig. 2. Heterozygous white-eyed crimson pink (*PW*).
Fig. 3. Crimson, white-eyed (*RP*).
Fig. 4. Mauve-purple (*MP*).
Fig. 5. Magenta-purple (*PL*).
Fig. 6. Pink-purple eye (*PP*).
Fig. 7. Red-blue eye (*RB*).
Fig. 8. Blue-pink (PP_2C).
Fig. 9. Red (*R*).
Fig. 10. Crimson-purple (*CPC*).
Fig. 11. Typical F_1 crimson with laciniated petal.
Fig. 12. Petals of small petalled form (cf. p. 16)
(*a*) white-eyed group.
(*b*) white group.
(*c*) dark-eyed group.

The letters given in parentheses are the short-hand designations used for the various colour forms in the text. Fuller descriptions of these forms, together with the factorial constitutions in so far as they have been determined, will be found on pp. 4—6.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

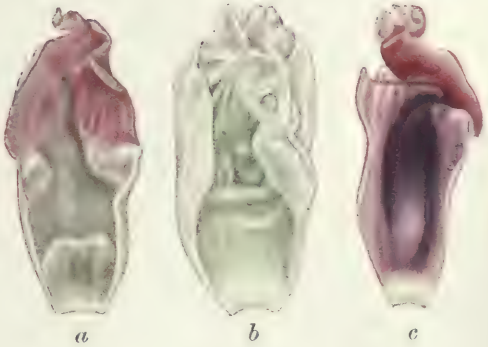
Fig. 7.

Fig. 8.

Fig. 9.

Fig. 11.

Fig. 10.



a *b* *c*

Fig. 12.

GENETIC STUDIES IN POTATOES.

THE INHERITANCE OF AN ABNORMAL HAULM TYPE.

By R. N. SALAMAN, M.A., M.D., *Cantab.*,
and J. W. LESLEY, M.A., *Cantab.*

(With Plates II—V.)

The varieties of potato haulm, whose anatomical and genetic relations are described in this paper, arose in the garden of one of us[1] at Barley, Hertfordshire. From the years 1906 to 1911 it had been the habit to train all the experimental seedlings raised in this garden up to horizontal wires placed 9″ and 18″ respectively above the ground. To these wires the haulms were loosely tied. In 1910 a line of plants, situated somewhat closely to a row of apple-trees, showed a uniform tendency to commit suicide, i.e., the stems would grow quite healthily till they attained a length of about 2 ft 3 ins by which time the last 9 inches of growth, instead of continuing its upright habit, would bend abruptly downwards and then the stem would get constricted or nipped at the point to which it was tied to the top wire. Other families, when not tied to supports and not in a shaded situation, often exhibited amongst their members examples who soon adopted a sprawling habit, but directly they had adopted this secondary position the terminals of the haulms would all take a fresh vertical direction. This variety will be discussed later.

Consideration of this family of plants gives rise to the conception of a potato whose haulm had an inherent desire to lie down rather than stand up. Later on in the season the true reason became manifest, viz., that the plants were tending to grow towards the light from which they were obscured by the apple-trees. The conception, however, remained, and by an extraordinary coincidence found a genuine basis in our cultures in 1911.

[1] R. N. Salaman.

In 1906 the variety Queen of the Valley was fertilized by Flourball. One of the F_1 seedlings was fertilized in 1908 by a seedling arising from a selfed ball of Bohemian Pearl. This gave rise to a family which was grown in 1909, one of whose units (*M*5 *B*35) was grown on in Barley in 1910, selfed in that year, and the seed sent to Cambridge University Farm for raising in 1911. The parent plant dropped out of culture.

After the seedlings had been planted out a short time it was obvious that the whole family were exhibiting a character which had not been met by us in any of our cultures. The stems, instead of standing erect, lay along the ground, creeping centrifugally so that as the plant became fully grown a varying area averaging not far short of a square yard would be closely covered by a fresh, healthy, green mass of foliage (Plate II, fig. 5). When handled the stems themselves felt like soft rubber tubes, which led to the natural inference that they simply lay on the ground because they could not stand up. The number of nodes and mode of branching was of the usual type, but the leaves all pressed upwards towards the light so that a well-developed plant of this type formed a flattened green cushion on the ground. The terminal leaflets turned towards the light, but the stems did not turn up vertically except at their extreme distal ends. This characteristic habit of growth has persisted, as will be detailed later, both in selfed cultures and in the vegetative successors grown from tubers. Nor indeed is this change of habit to be seen only after the stage of planting out. The seedlings when but 2″ in height show a tendency to collapse, and at a little later stage the whole seedling plant tends to flatten out (Plate III, fig. 1).

The habit of growth just described we have termed the "prostrate" one, and in this paper a "prostrate" stem means a stem from plants with this peculiar habit of growth.

One feature which was particularly obvious in 1911 (a year of great drought) was that the ground underlying all these plants was moist, whereas the surrounding area was hard and cracked. This feature will be referred to later.

A more or less upright character of the potato haulm is axiomatic amongst potato growers, who invariably look askance at any variety whose haulm tends to spread or flop. The stems of many varieties, however, on reaching maturity, tend to fall groundwards, but this is entirely different from the "prostrate" condition that has just been described. In these cases it is the excessive weight of the haulm and

perhaps loss of turgidity which causes the stems to become spreading or even to lie on the ground.

Before proceeding to an analysis of the anatomy of the variety it will be well to give an outline of the structure of an adult aerial potato stem. With so many distinct varieties of potato it is perhaps not surprising that we find somewhat divergent accounts of its anatomy and development.

The following account is, as far as we know, true of all cultivated varieties, and we shall therefore venture to call it the normal type.

The anatomical structure of the aerial stem of the potato normally resembles that of an ordinary herbaceous dicotyledon with the peculiarity common to nearly all Solanaceae of medullary phloem (Plate IV, fig. 1). The adult stem is roughly triangular in the internode and furnished with wings at the corners. A single outer layer sometimes beset with a few hairs constitutes the epidermis. The cortex is differentiated into narrow collenchymatous cells followed by a large celled inner zone with thin walls, while an innermost layer of slightly smaller cells ends in an endodermis often containing starch grains. The stele consists of a number of bundles united by secondary growth so as to form a continuous ring of phloem and xylem separated by the cambium and encircling a large pith containing islands of medullary phloem. The outer and inner phloem consists of parenchyma, fibres, sieve tubes and companion cells. As was noted above, the stem is for the most part roughly triangular, the sides of the triangle being flat or slightly convex. This shape is due to the presence of three main vascular bundles between which are much smaller bundles. The strands of fascicular cambium become connected by the interfascicular cambium arising in the medullary rays, and thus completed this tissue continues to form phloem outwards and xylem inwards. As a result an almost complete cylinder of vascular tissue is quickly formed. The protoxylem is readily recognized bordering the pith and consists of narrow elements. In the region of the actual bundles the secondary xylem, in addition to numerous narrower elements, contains large vessels especially in the three main bundles. The secondary xylem elements are thick-walled and are arranged in radial rows corresponding to their origin from the same meristematic cell. The interfascicular xylem consists almost exclusively of narrow thick-walled elements. Both the fascicular and the interfascicular xylem are lignified. The ground tissue of the pith is similar to that of the inner cortex. It contains no thick-walled elements with mechanical functions. The outer pith zone adjoining the xylem consists

of relatively small parenchymatous cells, towards the centre the cells are large with intercellular spaces. The medullary phloem occurs in little strands, especially in the small-celled pith near the protoxylem. These phloem strands originate in the medulla independently of the cambium, but Artschwager's[1] work has shown the interesting fact that these strands anastomose and at certain points are actually continuous with the ordinary outer phloem so that all the phloem of the potato plant forms one system. It may be added that cells containing fine crystal sand occur abundantly in the pith and to a less extent in the cortex.

Thus we see that the predominating mechanical tissue of the normal stem is the xylem and to a lesser extent the fibres which occur near the phloem and the collenchyma of the cortex. If there be any anatomical peculiarity of the stem affecting its rigidity and power to maintain an erect habit, it is to the xylem that we should first look.

A histological examination of the stems in the abnormal family grown at Cambridge[2] and of its descendants has brought a very definite peculiarity to light (Plate IV, fig. 2). The part of the adult stem taken was the internode of the basal part next to the ground, and the stains chiefly employed were Safranin, Phloroglucin and Schultze's solution. All thirty seedlings were examined and the following observations apply to them in common. While the fascicular cambium had formed much secondary phloem and xylem, in the interfascicular region or medullary rays between the bundles secondary tissues were either deficient or entirely wanting, and as the great bulk of the secondary tissue is xylem, the defective development of this tissue was most conspicuous.

Whilst the thick-walled lignified xylem of a normal stem forms a complete cylinder which may sometimes be thin or even broken for a very short space immediately below the insertion of a leaf, in the "prostrate" stem nothing approaching a continuous cylinder of wood is formed. The contrast is strikingly seen in pieces of stem which have decayed in water, or again after treatment with strong nitric acid so that all the tissues disappear except the lignified xylem (Plate V, fig. 1). In the normal stem the xylem forms a continuous system made up of fascicular groups connected by broad interfascicular bands, forming thus in transverse section a continuous ring of woody tissue; in the "prostrate," on the other hand, the wood rarely forms a continuous ring, and then only at isolated points in the length of the stem.

[1] Artschwager, *J. Agr. Res.* 1918, Vol. xiv. No. 6.

[2] The histological work in this paper has been done by J. W. Lesley.

Occasionally a slender band of thick-walled lignified elements is seen joining two of the three main bundles, sometimes two or even three thin bands of wood may be found at certain levels so that in the latter case a feeble but continuous ring of wood is formed at that level, but this condition is exceptional. Naturally enough the interfascicular cambium too is imperfectly developed. As a rule it extends along one or two sides of the stem and forms secondary xylem and phloem. Or, again, the interfascicular cambium may show signs of division only at certain points. The phloem is either scanty or only formed at intervals between the bundles, but wherever there is cambium phloem is found generally forming isolated groups according to the disposition of the cambium. The deficiency of phloem is less marked than that of xylem. Indeed it is the incomplete development of thick-walled lignified interfascicular xylem which is the characteristic anatomical feature of the "prostrate" plant.

The other tissues of the "prostrate" stem are the same as those of the normal type already described. The collenchyma is present in the outer cortex, only the larger cortical cells in the interfascicular region being somewhat rounder and less elongated tangentially owing to the lack of pressure from within which is exercised where much interfascicular xylem is formed. The internal phloem is fully developed, thus confirming its independence of the cambium.

The "prostrate" stem is more definitely triangular in shape than the upright, owing to the deficiency of interfascicular secondary growth.

The "prostrate" habit of growth is therefore associated with a deficiency of mechanical tissue which impairs its rigidity. For whilst the "upright" stem is practically a tube and owes its rigidity to a continuous cylinder of wood, the "prostrate" consists of a few strands connected, it is true, at certain points, but for the most part effectually isolated.

When once the pure breeding "prostrate" variety had been recognized, it became obvious that in our cultures certain plants we had met and which had been taken to be a "prostrate" variety were, though very similar in habit, not to be confounded with the true "prostrates" already described. Some of these plants and those which were subsequently extracted in pure culture at Cambridge resemble the "prostrate" in the lack of rigidity and rubber-like feel of their stems—a resemblance which is borne out in detail by their histological structure. They all differ, however, in one striking characteristic from the "prostrate" variety. The stems, once fallen to the ground, do not creep along horizontally as in the first variety, but after a short time the distal portions turn up

vertically. It is as if the plant wanted to stand up but couldn't, and after falling to the ground was making a fresh effort to regain the normal position, whereas in the other variety it has neither power nor desire to lead the "upright" life. The "prostrate" indeed not only falls to the ground but adapts its development in harmony with the new conditions.

For the second variety we suggest the term "procumbent" in contrast to the "prostrate" variety previously described.

Here then are two varieties sharing one character in common, deficiency of skéletal structure, but differing as we would suggest in their tropic reaction. Just as the stolon, the underground stem of the potato, has changed its tropic reaction, so has the aerial stem of the true "prostrate." Whilst the "prostrate" variety was only observed once in the history of our cultures and from that time specially bred, the "procumbent" has been noticed several times.

Genetic Relations.

The original family of "prostrate" plants came from the self-fertilization of a plant raised at Barley, Hertfordshire, in 1909[1]. This parent we shall refer to as *M*5 *B*35, following the original nomenclature. Its origin may in turn be traced back to three cultivated varieties—Flourball, Queen of the Valley and Bohemian Pearl. One would naturally expect to find the parent *M*5 *B*35 of this selfed family exhibiting the "prostrate" character. Unfortunately we have no evidence on this point, as stock of this plant was lost before attention had been drawn to the character in question. Among its ancestors again there is no record of the "prostrate" habit. Although it is possible that the character was missed it is on the whole improbable as *M*5 was certainly "upright," and we have never met with the "prostrate" character in any Bohemian Pearl (*B*) seedlings.

The results of breeding tests with "prostrate" plants will now be described. Most of the seed was obtained from unprotected flowers, although numerous attempts were made to obtain seed from flowers protected with parchment bags. As there is a risk of accidental pollination by insects—small it is true but certainly not negligible—we took the precaution of saving the seed of individual berries separately, for in this way we could more closely compare the results of similar pollinations. Of the thirty-one "prostrate" plants obtained from the selfing of *M*5 *B*35 and transplanted into the field in 1911 only two flowered. The season

[1] Salaman, R. N., *Journal of Genetics*, 1910, Vol. I. No. 1.

was extremely dry and hot, but twenty-seven of the thirty-one lived to produce tubers so that the mortality after transplanting out was low. In 1912 the whole family available was grown on from tubers. Twenty-six plants were grown on by vegetative propagation and exactly reproduced the "prostrate" habit of the preceding year. Twenty-one of them flowered, some having heliotrope, some white, flowers. Efforts were taken to secure selfed seed, and this time successfully; a few set seed naturally. Berries were obtained on six different individuals. A single berry was obtained from a protected and the remainder—about a dozen—from unprotected, generally hand pollinated, flowers. In 1913 the plants were again grown on from tubers and exhibited the same "prostrate" habit as in 1911 and 1912, and a little more selfed seed was obtained. Some of these original plants were again grown on vegetatively in 1914 for the fourth time. Many of them by this time were unhealthy and made poor growth, the leaves failing to develop perfectly. Whilst the healthy plants were again "prostrate" the unhealthy or "miffy" plants as they are termed did not completely reproduce the "prostrate" habit, although it is interesting to note that they retained the characteristic stem structure. This serves to show that it is not safe to classify "miffy" plants without examination of their stem anatomy. In fact, notwithstanding the ease with which the pliable stem of the "prostrate" plant is recognized, experience has shown us that it is best to confirm the observation by an examination of the stems. In most cases sections were examined under the microscope, but some only under a pocket lens after staining with phloroglucin.

The seed arising from the self fertilization of the "prostrates" was sown in 1913, 1914 and 1915, partly at Barley and partly at the John Innes Horticultural Institution, Merton. Some of the resulting families arose from a single ball, others from several balls, but all the families shown separately are independent and not mere fractional or duplicate sowings. The germination was fair, the mortality at planting out not unusually high and the plants quite healthy.

As Table I shows, nineteen independent families have been raised in three seasons from six individuals of the original "prostrate" family and grown in two different localities. With rare exceptions all the families consisted wholly of "prostrate" plants like the parents from which they arose and presented a striking contrast to adjacent "upright" plants (Plate II, fig. 1). Adding the figures together we have in all:

"Prostrate" 468.
"Upright" 3.

The three exceptions occurred in two families both arising from berries which set naturally. In the first case *M*5 *B*35, 1 a single very vigorous plant occurred with a "procumbent" habit of growth, one of the main stems especially turning upwards so as to render it quite distinct from the adjacent "prostrate" plants (Plate II, fig. 4). The stem of this,

TABLE I.

Serial No.	Name of Family	Where Grown	Method of Pollination		Habit: Upright	Habit: Prostrate
31 *B*	*M*5 *B*35, 1	Barley	By hand.	Unprotected	—	27
,,	,,	,,	Natural	... ...	—	23
31 *M*	,,	Merton*	,,	... ...	1	36
,,	,,	,,	By hand.	Unprotected	—	58
31 *B*	,,	Barley	,,	,,	—	18
32 *M*	*M*5 *B*35, 3	Merton	,,	,,	—	23
,,	,,	,,	,,	,,	—	20
33 *M*	*M*5 *B*35, 11	,,	,,	,,	—	21
,,	,,	,,	,,	,,	—	24
34 *B*	*M*5 *B*35, 23	Barley	,,	,,	—	33
,,	,,	,,	,,	Protected ...	—	16
,,	,,	,,	Natural	... ...	—	17
34 *M*	,,	Merton	,,	... ...	—	22
35 *M*	*M*5 *B*35, 24	,,	,,	... ...	—	26
35 *B*	,,	Barley	,,	... ...	—	5
36 *M*	*M*5 *B*35, 28 (1)	Merton	By hand.	Unprotected	—	38
36 *B*	,, (2)	Barley	Natural	... ...	—	14
36 *M*	,, (2)	Merton	,,	... ...	—	16
,,	,, (2)	,,	,,	... ...	2	31
			Totals	... ...	3	468

* John Innes Horticultural Inst., Merton.

however, proved to be structurally of the "prostrate" type. The two further exceptions occurred in one of the families grown from one of the naturally fertilized berries of *M*5 *B*35, 28. The stems were not of the "prostrate" variety; some of them bore tubers which did not conform to the family type, and there is very little doubt that these abnormal plants are due to foreign insect-borne pollen. We may safely conclude that the new variety breeds true. In 1913 three seedlings, offspring of one of the original family, produced four berries by natural and one by hand pollination under protection. The results from three of the families are anomalous as shown in Table II, but from unmistakable evidence deduced from the characters of the flowers, stolons and tubers we were able to show that the parent plant had been fertilized by a derivative of *S. edinense* which was growing near by and whose habit is "upright." The fact that in three of the families "prostrates" as well as "uprights" occur implies that besides the foreign dominant "upright" pollen the flower had been to some extent self-fertilized.

TABLE II.

Serial No.	Name of Family	Where Grown	Method of Pollination	Habit of Growth: Upright	Habit of Growth: Prostrate
31 *B* 7	*M* 5 *B* 35, 1, 7	Barley	Natural	12	—
31 *B* 18	*M* 5 *B* 35, 1, 18	Merton	,, ...	19	36
,,	,,	,,	,, ...	15	17
,,	,,	Barley	,, ...	11	3
34 *B* 45	*M* 5 *B* 35, 23, 45	,,	By hand. Protected	—	11
			Totals	57	67

It should be mentioned that since 1906 we have grown, in some cases repeatedly, selfed families of the many wild and trade varieties (see Table III). In no case have we found "prostrate" individuals, though

TABLE III.

Serial No.	Name of Family	Where Grown	Habit of Growth: Upright	Habit of Growth: Prostrate or Procumbent
Commercial:				
	President ...	Barley	91	—
1 *B*	Bohemian Pearl	,,	15	—
1 *M*	,, ,,	Merton	80	—
3 *B*	Flourball ...	Barley	28	—
3 *M*	,, ...	Merton	90	—
2 *B*	Blanchard ...	Barley	6	?2
19 *B*	Myatt's Ashleaf ...	,,	23	—
101 *B*	Blanchard ...	,,	15	—
108 *B*	Early Regent ...	,,	4	—
147 *B*	Port Wine Kidney	,,	10	—
Wild:				
151 *B*	*S. edinense* P. Berth	,,	9	—
,,	,, ...	,,	10	—
39 *B*	*S. tuberosum* ...	,,	24	—
211 *B*	*S. tub.* Herb. ...	,,	6	—
212 *B*	*S. tub.* Sander. ...	,,	4	—
213 *B*	*S. tub.* Vermont. ...	,,	38	—

it is only since 1911 that our attention has been closely drawn towards this character. In the case of Blanchard two seedlings were recorded as doubtful "prostrates," but no examination was made of their stems.

In addition to the above wild varieties *S. maglia* and *S. commersonii* have been grown for many years, but no selfed berries have been obtained: the habit of both parents is "upright."

In consideration of the evidence so far detailed, it may be accepted that the "prostrate" variety not only breeds true but reproduces itself without variation through the buds of its tubers.

F_1.

Twenty-three F_1 families in all have been raised from crosses of the type Upright × Prostrate and Prostrate × Upright, including one reciprocal cross. Fifteen out of the twenty-three were from flowers

protected in bags, whilst four members of the original "prostrate" family and sixteen different "upright" varieties were used as parents in the crosses.

The upright varieties include two ordinary cultivated varieties, the remainder being of varied origin, derived partly from cultivated varieties, partly from the Congo potato and partly from *S. edinense*. Although differing somewhat in their habit of growth in the same sense that ordinary cultivated varieties differ, they were all unmistakably "uprights" and their stems developed an abundance of secondary wood. The four "prostrates" used in these crosses were all true to type and identical in habit of growth and anatomical structure.

Evidence as to the genetic constitution of the prostrates has already been given. Selfed families have also been raised from twelve of the sixteen "upright" parents—from some of these two or three such families. In no case has a single true "prostrate" plant occurred. From the remaining four no selfed families have been obtained, three of them indeed produce no good pollen and the fourth could not be induced to set selfed seed, so that we must look to the evidence from crosses with other "uprights." This evidence, as far as it goes, confirms that obtained from selfing, for in no case have these "upright" plants when crossed with other "uprights" given rise to any "prostrate" plants. Two "upright" parents remain, however, which have neither given selfed families nor entered into any other crosses.

The results from the crossing of "upright" and "prostrate" plants (F_1) are shown in Table IV.

On the whole the plants were remarkably healthy and luxuriant, the mortality at transplanting unusually low. Twenty out of the twenty-three of the families, including all those from protected flowers, consisted entirely of uprights. Some of the plants were quite erect, the majority more or less spreading as the season advanced, but except in three families all were decidedly "upright."

The anatomical structure of the stems of a large number, in some cases of complete F_1 families, was examined by section and several by the method of maceration. While in some plants the interfascicular wood was thin and in others absent in patches, especially just below the leaf insertion, in the great majority there was strong interfascicular secondary growth. None showed the extensive deficiency and isolated strands of wood characteristic of "prostrates."

In three families, however, derived from two distinct "prostrate" and three distinct "upright" parents, a small proportion of true "prostrate"

TABLE IV.

A. *Upright* × *Prostrate.*

Serial No.	Name of Family	Where Grown	Method of Pollination		Habit of Growth: Upright	Habit of Growth: Prostrate
17 *M*	*K* 6[5] 104 × *ER* 28 × *M* 5 *B* 35, 23	Merton	By hand.	Unprotected	115	—
58 *B*	Kew 2 × *RR* 2 × *M* 5 *B* 35, 1	Barley	,,	Protected ...	12	—
59 *B*	Kew 2 × *RR* 2 × *M* 5 *B* 35, 23	,,	,,	,, ...	49	—
,, *M*	,, × ,,	Merton	,,	,, ...	65	—
61 *B*	Kew 2 × *RR* 12 × *M* 5 *B* 35, 28 [1]	Barley	,,	,, ...	70	—
74 *B*	Kew 19 × *ER* 1*b* × *M* 5 *B* 35, 1	,,	,,	,, ...	3	—
,, *M*	,, × ,,	Merton	,,	,, ...	55	—
78 *M*	Kew 19 × *ER* 8 × *M* 5 *B* 35, 1	,,	,,	,, ...	7	—
,, *M*	,, × ,,	,,	,,	Unprotected	22	—
81 *M*	Kew 19 × *M* 5 *B* 35, 23	,,	,,	,, ...	79	6
118 *B*	*Fb* 165 × *ER* 15 × *M* 5 *B* 35, 1	Barley	,,	Protected ...	32	—
123 *B*	*Fb* 165 × *ER* 65 × *M* 5 *B* 35, 1	,,	,,	,, ...	35	—
127 *B*	*J* 2 *RR* 11, 40, 48 × *M* 5 *B* 35, 28	,,	,,	,, ...	39	—
150 *B*	Up-to-date × *M* 5 *B* 35, 28	,,	,,	Unprotected	13	—
172 *B*	Kew 19 [2] 9 × *M* 5 *B* 35, 1	,,	,,	Protected ...	12	—
,,	,, × ,,	,,	,,	,, ...	12	—
168 *B*	Kew 2 × *ER*[1] 63, 46 × *M*5 *B* 35, 1	,,	,,	,, ...	30	—
204 *B*	*K* 15 × *M* 5 *B* 35, 1	,,	,,	,, ...	29	—
215 *B*	Kew 2 × *ER*[1] 63, 148 × *M*5 *B*35, 23	,,	,,	,, ...	54	—
217 *B*	Kew 19 × *M* 5 *B* 35, 1	,,	,,	,, ...	11	—

B. *Prostrate* × *Upright.*

38 *M*	*M* 5 *B* 35, 23 × *M* 5 *B* 32 × *Fb* 26	Merton	By hand.	Unprotected	39	6
145 *M*	*M* 5 *B* 35, 23 × Kew 2 × *ER*[1] 63, 148	,,	,,	,, ...	40	—
146 *M*	*M* 5 *B* 35, 24 × *Fb*	,,	,,	,, ...	91	1
			Totals	... ...	914	13

plants occurred. Two of these families arose from a cross of the type "prostrate" × "upright," the third from the reverse cross, and in each case there is a small but definite proportion of "prostrates." Two of the "upright" parents (Kew 19 and Flourball) have been selfed and yielded spreading but no "prostrate" progeny, but of the third, *M*5 *B*32 × *Fb* 26, we know nothing of its decendants.

Although some of the families are small, yet taken together the figures

"Upright" 914,
"Prostrate" 13,

show clearly that the "upright" character behaves as a dominant and the "prostrate" as a recessive.

The incomplete dominance observed in the three F_1 families out of the total twenty-three may possibly be due to faulty technique, seeing it has occurred only amongst those crosses which were not protected by bags. On the other hand, there is good reason to expect that our "upright" parents are not all of the same genetic composition as regards the factors for "prostrate," and in this relation it is particularly inter-

esting to note that the family in which the greatest number of recessives appear is *M*5 *B*35, 23 × *M*5 *B*32 × *Fb* 26—a cross in which the male is of the same kin as the "prostrate" female parent.

The reciprocal cross gives identical results, the "upright" being completely dominant. It is also of interest as being one of the very few reciprocal crosses which have been effected in potatoes.

F_2.

The evidence thus far has shown that the "prostrate" character behaves as a recessive. A considerable number of families were obtained from F_1 plants in the first or later years of their life, many of them from protected flowers. Only one F_2 was obtained from a "prostrate" × "upright," and this showed no peculiarity. The results are tabulated in Table V.

TABLE V.

F_2 from Upright × Prostrate.

Serial No.	Name of Family	Where Grown	Method of Pollination	Habit of Growth: Upright	Habit of Growth: Prostrate
17 *M*, 76	*K* 6[5] 104 × *ER* 28 × *M* 5 *B* 35, 23	Merton	By hand. Protected ...	12	1
59 *M*, 17	Kew 2 × *RR* 2 × *M* 5 *B* 35, 23, 17	,,	Natural	20	—
74 *M*, 18	Kew 19 × *ER* 1 *b* × *M* 5 *B* 35, 1, 18	,,	,, ...	42	1
74 *B*, 2	,, × ,, 2	Barley	,, ...	28	—
74 *M*, 38	,, × ,, 38	Merton	,, ...	26	—
78 *M*, 2	Kew 19 × *ER* 8 × *M* 5 *B* 35, 1	,,	By hand. Unprotected	10	1
,,	,, × ,,	,,	,, ,,	10	—
81 *M*, 58	Kew 19 × *M*5 *B* 35, 23, 58	,,	Natural	8	—
,,	,, × ,,	,,	,, ...	7	—
58 *B*, 3	Kew 2 × *RR* 2 × *M* 5 *B* 35, 1, 3	Barley	,, ...	10	—
,, 8	,, × ,, 8	,,	,, ...	17	—
61 *B*, 94	Kew 2 × *RR* 12 × *M* 5 *B* 35, 28[(1)], 94	,,	,, ...	35	—
123 *B*, 2	*Fb* 165 × *ER* 65 × *M* 5 *B* 35, 1, 2	,,	By hand. Protected ...	81	6
,, 9	,, × ,, 9	,,	,, ,, ...	90	—
,, 16	,, × ,, 16	,,	,, ,, ...	96	—
,, 24	,, × ,, 24	,,	,, ,, ...	84	—
,, 29	,, × ,, 29	,,	,, ,, ...	58	—
127 *B*, 2	*J* 2 × *RR* 11, 40, 48 × *M* 5 *B* 35, 28, 2	,,	,, ,, ...	45	2
145 *B*, 13	*M*5 *B* 35, 23 × Kew 2 *ER*[1] 63, 148, 13	,,	,, ,, ...	62	—
215 *B*, 3	Kew 2 *ER*[1] 63, 148 × *M* 5 *B* 35, 23, 3	,,	,, ,, ...	72	1
		,,	,, ,, ...	13	1
			Totals	826	13

The first point to note is the very small number of "prostrates" which reappear. As in F_1 there was a large number of spreading plants which in the field were occasionally difficult to distinguish from "prostrates." In such cases the anatomical test was applied with decisive results. In a few plants slight defects in the development of secondary wood were observed as in some of the F_1, but the true

"prostrates" formed a distinct class combining the creeping habit with the extensive deficiency of interfascicular secondary growth characteristic of the type.

The families are derived from nine distinct P_1 "upright" and three distinct P_1 "prostrate" parents. Seven out of the twenty-one families contain a small proportion of "prostrates," while the remaining fourteen contain only upright plants. The proportions certainly do not point to a single factor difference in any. On the contrary, all the larger families clearly show that the "prostrate" and "upright" differ in more than one factor. The five F_2 families from the same cross (123*B*) are especially remarkable. One contains "prostrates" in a proportion approaching $\frac{1}{16}$, whilst the others contain none. If we put them together they give:

"Upright" 409 to "Prostrate" 6,

nearly a 63:1 ratio, but on this basis the figures in 123*B*, 2 are not within the limits of simple sampling thus:—

Actual Numbers	Calculated on 63 : 1	3σ
81 : 6	85·64 : 1·36	3·47

so that the deviation is about four times the standard error. Of the other families 145*B*, 13 suggests a 63:1 ratio, as do the two families from 127*B*.

Taken all together the figures are:

"Upright" 826,
"Prostrate" 13,

or approximately 63:1, but of course it is not certain that this summation is legitimate.

F_3.

F_2 plants from crosses between "uprights" and "prostrates" were selfed either in the first year or when grown on from tubers. All the seed obtained was from protected flowers, in fact the entire succession of F_1 and F_2 families in this series were from similarly bagged seed. Table VI shows the results obtained in these F_3 families.

Now if, as the proportion in F_2 led us to suppose, the "upright" and "prostrate" parents differ in three factors, any one of which in a single dose gives "uprightness," F_2 "uprights" when selfed should prove to be of four different kinds; they will consist of pure "uprights" and of impure "uprights" of three distinct kinds, each yielding "uprights" and "prostrates" in different proportions. More than half ($\frac{37}{63}$) will be pure "uprights," the remainder ($\frac{26}{63}$) impure; of these latter nearly half ($\frac{12}{26}$)

will yield "uprights" and "prostrates" in the ratio of 15 : 1, about one-third ($\frac{8}{26}$) should give 63 : 1 and the remainder ($\frac{6}{26}$) 3 : 1.

In Table VI not only is segregation seen to take place but it is evident that different impure "upright" individuals give rise to different

TABLE VI.

Serial No.	Name of Family	Where Grown	Method of Pollination	Habit of Growth: Upright	Habit of Growth: Prostrate
123 *B*, 2, 16	*Fb* 165 × *ER* 65 × *M*5 *B* 35, 1, 2, 16	Barley	By hand.	Protected	10	3
123 *B*, 16, 32	,, × ,, 16, 32	,,	,,	,,	35	1
123 *B*, 24, 19	,, × ,, 24, 19	,,	,,	,,	38	3
,, 23	,, × ,, ,, 23	,,	,,	,,	24	—
,, 65	,, × ,, ,, 65	,,	,,	,,	26	—
127 *B*, 2, 32	*J*2 × *RR* 11, 40, 48 × *M*5 *B* 35, 28, 2, 32	,,	,,	,,	9	—
,, 34	,, × ,, ,, 34	,,	,,	,,	7	1

families of "uprights" and "prostrates" bearing ratios at least as diverse as 3 : 1, 15 : 1 and 63 : 1. The numbers both of the families grown and that of plants which compose them are too small to determine accurately the rather complex relations of the "prostrate" family.

In view of the very small proportion of "prostrates" which occur larger F_2 and F_3 families are required, but the results at present point to the conclusion that at least three factors are at work. In view of this the discovery of but one original recessive "prostrate" plant in one family among many hundreds involving the examination of about fifty thousand seedlings is not surprising.

As to the intermediate type we have already referred to the "procumbent," besides which there are many plants which though counted amongst the "uprights" because they neither crept nor were deficient in interfascicular wood yet differed from the fully "upright" plant, some tending to fall to the ground at an early age whilst others grew in a bush-like manner. There are certainly many intermediate stages between the true "upright" and the true "prostrate" besides the "procumbent," but there are not sufficient criteria to justify us in making a more critical classification.

The Procumbent Variety.

It has already been mentioned that an intermediate form has been found in our cultures forming a link between the "upright" and the "prostrate" varieties. This type was recognized as an abnormal one in 1910 and photographed in 1911, but its true significance was not recognized till 1912 when in a family (*J*2 × *RR*11, 40) in which the Congo, Flourball and Reading Russet varieties take part, a certain proportion of

plants was noticed to resemble the "prostrate" variety differing however in one characteristic manner, viz., the terminal part of the flaccid stem instead of lying flat on the ground turns boldly upward. As growth proceeds the proximal part of the stem assumes the horizontal position. Anatomically the stem exhibits the same structure as does the "prostrate" and shows the same extensive deficiency of interfascicular secondary growth. At present the evidence is too incomplete to establish the genetic relationship of the "procumbent" type of haulm, though we have reason to think that it is a pure recessive differing in but one factor from the "upright."

We may represent the case then as follows: The "upright" potato has undergone at least two modifications. The first affects the stem structure and is seen in the "procumbent" type. The second modification besides that of stem structure has led to a further change, possibly of tropic sensibility, and results in the "prostrate" habit of growth. Whether the tropic modification occurs without the anatomical we cannot say, although we believe that the anatomical can exist apart from the tropic as is shown in the "procumbent" type. It is to be regretted that there are at present no crosses between the "procumbent" and "prostrate" families.

Certain considerations naturally arise in connexion with the foregoing results. One question is whether the anatomical peculiarity of the "prostrate" and "procumbent" plants has any physiological effect which we can detect. The deficiency of phloem does not amount to much as the medullary phloem is not affected, but the deficiency of xylem is very pronounced though the large vessels are well represented. The water supply to the leaves might be reduced and so affect the metabolic processes and indirectly tuber formation. Experience shows that "prostrate" and "procumbent" plants give individual crops fully as large as "uprights," indeed, in 1911, an exceptionally dry summer, the "prostrates" suffered less from drought and gave remarkably good crops.

Again the failure of the interfascicular cambium does not appear to be associated with poor tuber formation or as far as we know with defective cell division elsewhere in the plant, so that this remarkable loss of the power to divide appears to be confined to the interfascicular region of the aerial stem.

Indeed no correlation between habit of growth, incidence of Phytophthera blight or other characters has hitherto come to light. It is an interesting fact that the pure "prostrate" families showed a most remarkable uniformity not only in habit of growth but in other characters

such as foliage, tuber shape, stem colour and stolon length and, in respect to certain families, of flower colour also. No doubt this is merely due to their homozygous constitution resulting from self-fertilization and isolation.

The "prostrate" plant covers a large area of ground with its dense foliage and it has been found that the soil so covered is always damp, so that even in the drought of 1911 these plants were always moist at the roots and maintained a high cropping rate. It has occurred to us that such a habit might prove of value in sub-tropical countries and arrangements for experimenting with the variety in Palestine had been made in 1913 with the late Dr A. Aaronsohn of the Haifa Technical Institute. The materials sent, however, were all destroyed by the Turks, together with the Institute, during the war. The tragic death of Dr Aaronsohn, to whose memory we would like to pay homage, is not merely a personal loss but one that is irreparable in the sphere of agricultural research in the East.

The foregoing results may now be briefly summarized. Two varieties differing in habit of growth have arisen from the normal potato plant. An inherited variation has been brought to light which affects the anatomical structure and rigidity of the stem and consequently the habit of growth.

The "prostrate" variety breeds true. The genetic relations as far as they have been elucidated show that the "prostrate" differs from the "upright" in at least two and probably in three factors, and finally that the "prostrate" habit is not correlated with other characters.

In conclusion the authors wish to acknowledge the valuable assistance given by Miss E. Hagger in continuing at Barley the experiments during the war and making many valuable observations. They also desire to tender their thanks to Mr Bateson and Professor Biffen for permitting some of the cultures to be grown in their experimental grounds and for the unfailing encouragement they have afforded them.

Fig. 1.

Fig. 2.

Fig. 3.

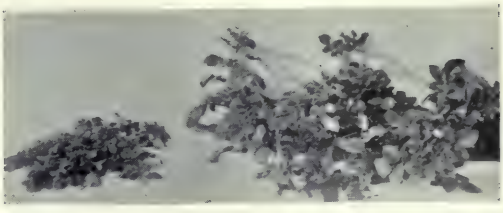
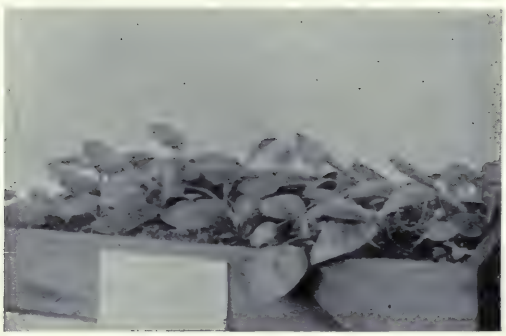


Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.



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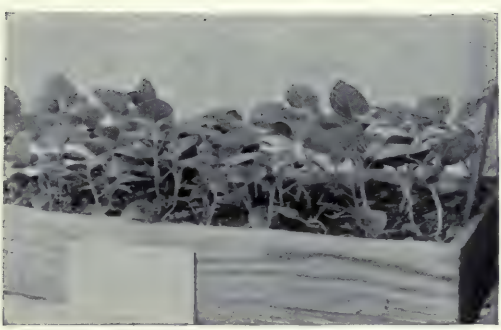

Fig. 2.

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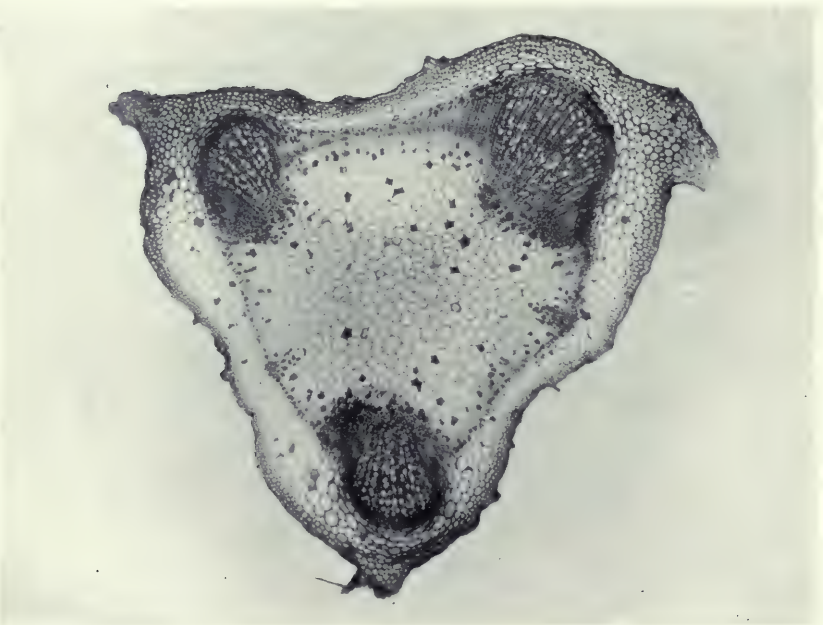

Fig. 2.

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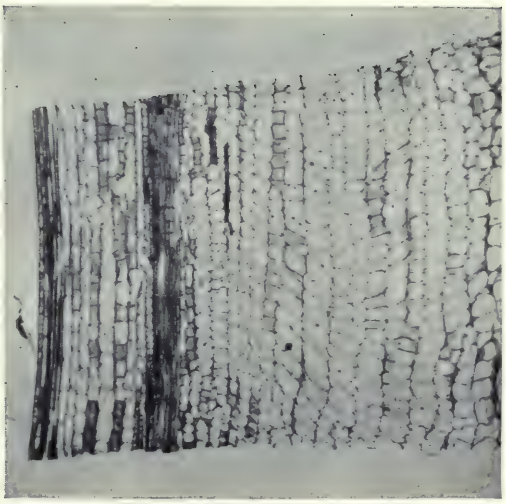
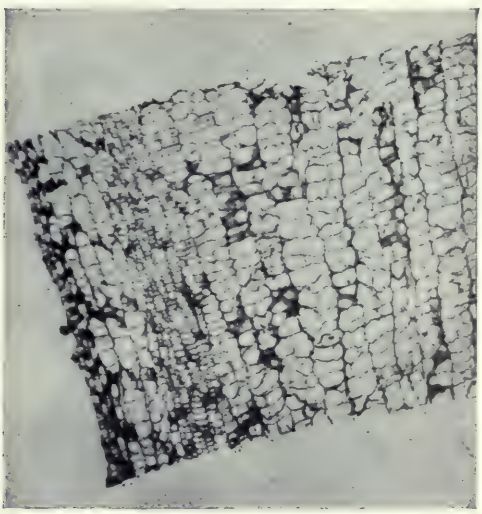


(1)



(2)

Fig. 2.

DESCRIPTION OF PLATES II—V.

PLATE II.

Fig. 1. Pure "prostrate" families—*M* 5 *B* 35, 1.
A row of "upright" plants is seen in the background and part of a row on the extreme right.

Fig. 2. The original "prostrate" family *M* 5 *B* 35 as grown in Cambridge.

Fig. 3. "Upright" and "prostrate" plants in an F_2 family from "prostrate" × "upright."

Fig. 4. Left, a typical "prostrate"; right, a "procumbent" plant in the same family from a "prostrate" (*M* 5 *B* 35, 1) selfed.

Fig. 5. "Prostrate" plant.

Fig. 6. "Procumbent" plant from "procumbent" selfed.

Fig. 7. "Procumbent" plants segregating out in a heterozygous family. (Kew $A \times K^6$ 128.) To the left an "upright" followed by three "procumbents," on the extreme right a bushy "upright" plant.

PLATE III.

Fig. 1. Pure "prostrate" family, *M* 5 *B* 35, 1.
The seedlings have already collapsed and begun to adopt the characteristic habit.

Fig. 2. F_1. *Fb* 165 × *ER* 65 × *M*5 *B* 35, 1. ("Upright" × "prostrate.")
The plants are as "upright" as those in fig. 3, illustrating dominance of "upright" over "prostrate."

Fig. 3. F_1 between two "uprights." (Early Puritan × *Fb* 165 × *ER* 65.)

PLATE IV.

Fig. 1. Transverse section of adult aerial stem of an "upright" (Flourball derivative). Safranin: showing interfascicular xylem. × 15.

Fig. 2. Transverse section of adult aerial stem of "prostrate" localized and weak development of interfascicular xylem. × 15.

PLATE V.

Fig. 1. "Retted" stem of "prostrate" (*M* 5 *B* 35, 28) on the left and of "upright" (a Flourball derivative) on the right.

Fig. 2. Part of radial longitudinal section of interfascicular region of aerial stem of

(1) an "upright" (variety of "Up-to-date") showing strip of cambium and early secondary vascular tissue.

(2) a "prostrate" (seedling from *M* 5 *B* 35, 28).

No cambium or secondary vascular tissue. × 20.

SOME NOTES ON FRAGARIA.

By C. W. RICHARDSON.

(With Two text-figures.)

Flower colour. Continuing my experiments recorded in this Journal (Vol. VII, No. 3, May 1918 and Vol. III, No. 3, Feb. 1914) I selfed in 1918 a pink double F_2 of my cross double flowering *vesca* × single pink and obtained a family of 110 all pink doubles. The shade of pink varied but little and never reached the stage of crimson.

On going over the plants of a cross-back (*virginiana* × *chiloensis*) F_1 × *chiloensis lucida*, to trace the last remnant of *virginiana* (which I failed to do), I lighted on many pink flowers and one plant with partially crimson flowers of abnormal shape. In the bud the petals were green or edged with crimson, on opening they showed white splashes on a green ground which rapidly turned crimson. The stamens and carpels were malformed and the flowers sterile. The general effect of the crimson was to make the flowers resemble pear blossom though the old petals were darker in colour. Runners taken with every care from the parts of this plant yielding abnormal flowers have flowered perfectly normally this season (1919).

Now in the ordinary pink *vesca*, colour appears in the early bud stage and is passed on to its descendants: but this colour must not be confused with that arising from damage by rain or grit, which is of frequent occurrence. To this latter condition may now be added damage from abnormal weather conditions causing defective nourishment, as in the so-called "summer" of 1918.

Double flowering. As already stated my F_3 doubles from doubles were all double, none were of the single, extra petalled F_1 type. Any very strong flowering plant of a garden variety, or of a species cross, may give rise to plants with extra petalled or even slightly doubled flowers, but I have not found this condition constant, still less capable of transmission from parent to child. The real double, never quite perfect, arises from a lasting change in the sex organs.

Foliage. The leaflets of *chinensis* are much rounder than those of *virginiana*. Crossed with *grandiflora* ♂ it produces a mixed progeny, having leaflets ranging from nearly as round as those of *chinensis* to rather longer than *virginiana*. Grading them from the roundest to the longest I classified 39 such plants in five groups as follows:

Roundest				Longest
9	10	6	3	11

The first three groups (with 25 plants) could be called round and the other two (with 14 plants) could be called long.

Similarly graded in point of thickness of leaf, four groups were made:

Thickest			Thinnest
10	15	12	2

The long may be thick and the round thin.

The object of this experiment was to test *grandiflora* for mixed origin and the result, to my mind, is conclusive—*grandiflora* resembles *chiloensis* or *chinensis* more than *virginiana*, but it is evidently heterozygous, and has probably arisen as a cross and not as a sport from either of those forms.

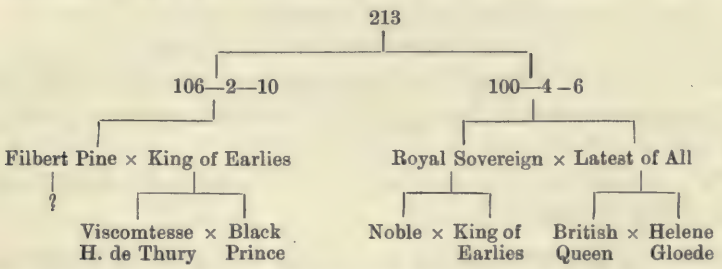
(*Chinensis* × *chiloensis*) F_1 ♀ × F_1 (Royal Sovereign × Givon's Late Prolific) (Plant family 211) gave F_1 plants with many leaflets, six in one case and frequently five, also normal trifoliate plants—13 normal, 11 abnormal—the numbers are small but point to equality. All the plants of this cross were very strong growing in every way. The foliage was dark in all cases. *Chinensis* ♀ × *elatior* ♂ (204) gave F_1 plants with very strong growing and light coloured foliage, so like *elatior* that if the cross had been made *elatior* ♀ × *chinensis* ♂ I would have repeated it, fearing some accident had taken place, but as all my *chinensis* plants are female, and cross backs with both parents show, in the small numbers at present to hand, unmistakable signs of segregation, I have no hesitation in accepting the cross as perfectly true—F_2's from selfed F_1's unobtainable up to now. *Chinensis* ♀ × *Daltoniana* (189) gave F_1 plants with large leaves of the *Daltoniana* type. F_2 unobtainable up to now.

F_2 plants from a cross (*chiloensis* × a garden variety) crossed back with another garden variety (208) still retain the excessive vigour of their foliage. The F_2 selected had leaves of slightly *chiloensis* type, and the cross back gave plants of the garden variety type in 45 cases out of 50.

Variegation in foliage. The lack of chlorophyll is such a fluctuating character in Fragaria that one is not attracted to it as a study, when

there are so many other plants giving more certain examples of the phenomena. Yet occasionally it is impossible to neglect a demonstration, such an one I found in my family 213 (of which more to come). Here last May, 40 plants were normal—6 appeared normal, 6 showed signs of variegation, 8 were variegated, 5 chosen as normal have grown well and remained normal. Five of the doubtfully variegated have shown variegation, the remaining doubtful plants have not grown well. This looks like 46 normal to 14 variegated, which may very well be 45 to 15, or 3 to 1. I found the fruit of some of the variegated plants was mottled, but other fruits on the same plants were not. The only clue I have to this "sporting" is the fact that some of my selfed Royal Sovereigns were variegated, and accordingly the descendants may carry this character.

Fruit-Flavour. In the pedigree given below I quote the parents of garden varieties as generally accepted and have no reason to doubt them. In any case they are not all important.



Filbert Pine selfed in 1909, out of 100 plants only 30 had fruit the quality of which could be estimated in 1911 and 1912; of these about 6 had fruit with fair or good flavour. I have since devised a scale in which a perfectly flavoured fruit would receive 12 marks and one with a very bad flavour 1 mark. Taking the well-known plant Royal Sovereign as an example, I would award it 8 marks; but a plant with no flavour would receive 6 marks. If this system had been in use I would in no case have given more than 8 and in many cases but 3. Filbert Pine is not a good plant as it is exceptionally liable to pests, but it has a good flavour—a 9 mark one may be.

King of the Earlies selfed in 1909, out of 100 plants only 3 had fruit the quality of which could be estimated in 1912. Some people think well of this plant, but I merely say " it is a matter of taste."

Royal Sovereign selfed in 1909, out of 130 (perhaps 150 as there were some little things which never grew out of the first leaf, early primitive,

stage) only 32 had fruit the quality of which was estimated in 1911 and 1912. On my present system of marking I would have classed these:

3 given 3, 2 given 4, 3 given 5, 2 given 6, 7 given 7, 12 given 8, 3 given 9.

The remaining 98 plants were bad or wanting in flavour.

Latest of All selfed in 1909, out of 75 plants 28 had fruit the quality of which was estimated, these would have been marked:

5 given 4, 13 given 5, 1 given 6, 1 given 7, 7 given 8, 1 given 9.

Of the remaining plants most would have been given 6. I have often wondered if this is as good a plant as British Queen; it is hardly as good in flavour, but it has other good qualities.

From the above it will be seen that the parent plants when selfed give very few plants of good flavour.

"*106.*" Out of 72 plants only 6 had fruit the quality of which could be estimated. Of these 106–2–10 had the best, probably better than either parent; it would have received 9 marks.

"*100.*" Out of 50 plants only 3 had fruit the quality of which was estimated. Of these 100–4–6 was quite the best, certainly better than either parent, and would have received 9 marks. So we have two parents recessive for flavour.

"*213.*" Plants from this cross made in 1914 and planted out in 1918 gave the following result, out of 37:

1 given 3, 3 given 4, 6 given 5, 3 given 6, 5 given 7, 15 given 8, 2 given 9, 2 given rather over 9.

The above records point to the fact that flavour is a character built up of several characters, which may depend on several factors. Some years ago Miss F. Durham very kindly extracted a volatile essential oil from strawberries, and helped me in other experimental work of this nature, which satisfied me that an essence was present in strawberries. No doubt the amount of sugar in the plant is a factor on the + side in flavour, but I have not ventured to go into this up to the present. I am strongly disposed to think that all ripe strawberries have just begun to ferment, as flavour appears to be stronger in fruit verging on the over ripe stage. I must add many strawberries have a horrible flavour—some friends I "tried them on" said they resembled cold mutton that had slightly turned. This is probably due to the presence of a flavour and absence of all sweetness, though unripe fruit is often of good flavour yet slightly sharp. Before leaving this subject I should like to add—I no

longer believe size is a deterrent to flavour. They are both very complex characters, so the chances of uniting them are small. Added to this, they are not the only characters the fruit grower wants when he sends his baskets by train etc. to market.

Size of Fruit. Till this year I have never had a family producing large fruit as the rule. From the family 211 (*chinensis* × *chiloensis*) × garden strain I obtained fruit displacing 8, 10, 10, 11, 12, 12, 12, 12, 12·5, 13, 13·5, 14, 15, 16, 16·5, 17, 18, 18, 19, 20, 22, 24, and 28 c.c. of water. The fruit measured was in no case the largest borne by any plant. The largest fruit measured was slightly slug-eaten and displaced 32 c.c. of water. Other families of garden varieties, growing alongside this, gave fruit of 18 to 22 c.c. as the largest, and small fruits of 5 to 8 were very frequent. This 211 family chiefly consists of hard-growing, multi-fruit-stemmed and very heavy cropping plants, but the fruit has next to no flavour, and its shape may be any form of carpet bag, clenched fist, cock's comb or globe, with seeds yellow or dark crimson to nearly black.

The cross (*chinensis* × *Daltoniana*) has only given me three seeds; but it is worth noting that the flowers are borne in trusses and not singly as in *Daltoniana*, following in this the cross (*Daltoniana* × *vesca*).

The cross (*chinensis* × *elatior*) has not given me any seed up to now, but I have reason to believe it may do so in the future. The flowers are described below.

Runners. There seems to be a morphological resemblance between runners and flower stems. Last autumn I had several cases of runners flowering before they made foliage or rooted; runners from these have behaved normally. This summer a plant from the *chinensis* × *elatior* cross produced flowers with very long stems, and from the flower trusses runners grew (Fig. 1). This cross in 1915 gave one plant which continued from December to March or April to send out fresh trusses from the trusses which had flowered. (I have no photograph of this unfortunately.)

Sex. *Chinensis* ♀ × *Daltoniana.* Out of 22 well-established plants 7 ♀, 7 ♂ or ☿ and 8 *blind* plants. Most of the plants that flowered had done so the previous year, but not one of the *blind* plants had.

Chinensis ♀ × *elatior* ♂. Out of 25 plants which had been three summers in the ground only nine have flowered. Fig. 2 shows five types of flowers from five plants. Following my rule of classifying plants as female *or* males and hermaphrodites I count these 1 female and 8 male or hermaphrodite. The pollen is good in the latter class.

Fig. 1. *Chinensis* × *elatior*.

(*Chinensis* × *chiloensis*) × *Garden* (211). 24 plants gave 12 ♀, 12 ♂ or ☿. The number of plants in all these cases is small, but I give the figures to add to my former list 183 ♀, 155 ♂ or ☿. So things now stand 203 ♀, 173 ♂ or ☿.

Compared with the normal, with three leaflets, *Monophylla* is degenerate in foliage and frequently in flower: on the other hand crosses between species frequently give multi-foliate plants which seems to point to the fact that the three leaf plant has not fully developed; the flowers of such plants are sometimes quite perfect, e.g. the *chinensis* × garden cross, and sometimes degenerate, e.g. the *virginiana* × *vesca*

Fig. 2.

cross where very few set seed and there is little pollen, but what there is seems good. *Chinensis* × F_2 (*virginiana* × *vesca*) set seed freely, which has germinated very well. One may expect a strong race of plants from a cross with *chinensis* or *chiloensis*, and it is more than likely such a race will produce five or even six leaflets on the end of the leaf stalk and two more leaflets on the stalk some three inches lower down. With plants breeding true for flavour it will be possible to make these robust crosses produce fruit of good flavour. Such crosses produce fruit with large seeds, and if they produce these seeds in large numbers the fruit will be large and of good form. The actual shape can no doubt be arranged by crossing with plants of the shape desired; there is some evidence that long is dominant to round, but at present this is a little confused by

fruit-doubling or trebling and taking a spade-like or cock's comb shape. There is some difficulty at present in breeding pure hermaphrodites as some free flowering plants set very little fruit, and plants from seed of such fruit are far from uniform in the amount of fruit they set. I had *virginiana* and Hautbois males which never set seed whether grown indoors or out.

Last Autumn, Winter and Spring seed was germinated under glass: (1) exposed to sunlight, (2) under one thickness of thick white paper, (3) in the dark. The results of many tests were so conflicting that the satisfactory conclusion was come to that light has very little definite effect on the germination of strawberry seed. Whilst engaged on these experiments I found seed from the same fruit might take five days or ten weeks to germinate, as a rule the larger the seed the sooner it germinated, but this was not invariably the case.

In conclusion I am very glad once again to be able to thank the John Innes Horticultural Institution for the facilities afforded me in carrying on my experimental work. A season which has demanded more watering than is generally required in this country has been helpful in encouraging the production of pollen, but has largely reduced the size of fruit and checked the growth of shallow rooting varieties.

NOTE. The plants named "*chinensis*" in these and other notes published in this Journal were obtained from the Royal Botanic Gardens, Kew, in 1910, where the parent plants are still to be found. This name does not appear in *The Index Kewensis*, but is used in the private records at Kew where reference is made to these plants. The plants are very hairy, smaller, lighter in colour and less shiny than *chiloensis*. I would class them as a subspecies of *chiloensis*, whether they came from China or not. Plants crossed with known species gave in the F_1 families individuals which appeared to be alike in all points except six. Unfortunately all the plants are ♀ so I have not been able to self.

C. W. R.

NOTE ON A CASE OF LINKAGE IN *PARATETTIX*.

By J. B. S. HALDANE, M.A.,
Fellow of New College, Oxford.

Nabours (1) has shown that the various colour patterns in the grouse-locust, *Paratettix texanus*, are due to a series of Mendelian factors *A*, *B*, *C*, *D*, *E*, *F*, *H*, *I*, *J*, *L*, *N*, *P*, *Q*, and *S*, which are multiple allelomorphs or else very intensely repelled (this repulsion has been changed to coupling for one pair only), and a factor Θ allelomorphic to its absence only. Nabours regarded this factor as being inherited quite independently of the others. A close examination of his data shows however that in spermatogenesis Θ and any of the other factors exhibit linkage with a cross-over value of about 24 °/₀ or nearly that of the 1 : 3 : 3 : 1 system, whilst in oögenesis they exhibit a small linkage, the cross-over value being about 46 °/₀.

The notation used below is that of Nabours, except that the absence of Θ is represented in the usual manner by θ, and a zygote known to have been formed from gametes carrying $A\Theta$ and $B\theta$ is represented by $A\Theta . B\theta$, and so on. In all matings the composition of the father is shown on the left. In Table I are shown the results of Nabours' matings in which the male was doubly heterozygous, and his composition can be determined from that of his parents. Those matings where such a male was mated to a female carrying the same factors are excluded, as we have here no means of determining how much of the observed linkage was due to each parent. Column 1 gives the composition of the parents, Column 2 the number in Nabours' catalogue, Column 3 the total number of young produced, and Column 4 the number of these which were due to "crossing-over" or rearrangement of the factors during spermatogenesis.

For example in Mating 180 the male was of composition $A\theta . B\Theta$, since his parents were $AB\,\Theta\theta$ and $A\theta . S\theta$. When mated to the female $A\theta . S\theta$ he begot:

	45 $A\theta . A\theta$	and	$A\theta . S\theta$,
	17 $B\theta . A\theta$	„	$B\theta . S\theta$,
	17 $A\Theta . A\theta$	„	$A\Theta . S\theta$,
and	37 $B\Theta . A\theta$	„	$B\Theta . S\theta$.

Hence out of 116 spermatozoa he must have produced:

45 $A\theta$, 17 $B\theta$, 17 $A\Theta$, 37 $B\Theta$,

or 34 cross-overs out of 116. When the mother was also heterozygous for Θ certain classes of zygotes could not be distinguished, and hence only those classes are considered which enable us to measure the linkage directly. Thus in mating 178 all the $A\theta B\theta$ and $B\theta F\theta$ zygotes were formed from non-cross-over spermatozoa, whilst $A\theta A\theta$ or $A\theta F\theta$ would have been due to crossing-over.

TABLE I.

Male doubly heterozygous.

Mating	Number	Totals	Cross-overs
$A\Theta . B\theta \times A\Theta . F\theta$	178	7	0
$A\theta . B\Theta \times A\theta . S\theta$	180	116	34
$A\theta . C\Theta \times B\theta . C\theta$	187	57	19
$B\Theta . C\theta \times B\theta . C\theta$	231	21	3
$B\Theta . C\theta \times B\theta . P\theta$	260	42	7
$B\theta . C\Theta \times B\Theta . B\theta$	237	1	0
$B\theta . C\Theta \times B\theta . C\theta$	229, 230	104	30
$B\theta . C\Theta \times B\theta . S\theta$	263	45	13
$B\theta . C\Theta \times C\theta . F\theta$	266	94	25
$B\theta . C\Theta \times C\theta . P\theta$	271, 272, 273	194	38
$B\theta . F\Theta \times B\Theta . B\theta$	294	9	2
$B\theta . F\Theta \times B\theta . C\theta$	295	40	15
$B\theta . F\Theta \times B\theta . E\theta$	296, 297	63	8
$B\theta . F\Theta \times C\theta . C\theta$	309	60	10
$B\theta . F\Theta \times C\Theta . F\theta$	312	30	4
$B\theta . F\Theta \times E\theta . F\Theta$	313	9	1
$B\Theta . P\theta \times C\theta . P\theta$	337	38	7
$C\Theta . E\theta \times C\theta . E\theta$	372	10	1
$C\theta . F\Theta \times A\theta . C\theta$	370	104	22
$C\theta . F\Theta \times A\theta . J\theta$	373	14	2
$C\theta . F\Theta \times B\Theta . C\theta$	268	32	12
$C\theta . F\Theta \times B\theta . C\theta$	283	8	1
$C\theta . F\Theta \times B\theta . F\Theta$	310, 311	47	17
$C\theta . F\Theta \times C\Theta . C\theta$	375	19	4
$C\theta . F\Theta \times C\theta . F\theta$	376, 377, 378	160	38
$C\theta . F\Theta \times F\Theta . J\theta$	389	8	3
$C\Theta . P\theta \times B\theta . C\Theta$	398	4	1
$C\Theta . S\theta \times B\theta . B\theta$	425	32	11
$C\Theta . S\theta \times B\Theta . C\theta$	426	5	1
$C\Theta . S\theta \times C\Theta . C\theta$	417	4	1
$C\theta . S\Theta \times C\Theta . C\theta$	416	19	1
$E\theta . F\Theta \times B\theta . C\theta$	430, 431	25	4
Totals		1421	335

Out of 1421 spermatozoa produced by the doubly heterozygous males 335, or 23·6 %, were due to crossing-over. The cross-over value appears to vary slightly with the pair of "multiple allelomorphs" concerned, but the only variation which is probably significant is as follows. Where one of the factors concerned is A, i.e. in matings 178, 180, and 187, the cross-overs number 53 out of 180, giving a cross-over value of $29{\cdot}4 \pm 2{\cdot}3$ %,

whilst for the other factors together the cross-over value is $22{\cdot}7 \pm 8\,\%$. The probable errors given are those due to random sampling only. The divergence may probably be explained on the following hypothesis, which is substantially that of Dexter (2). The factors *B*, *C*, *D*, etc. are incompletely dominant over their absence, which is represented by *A*, and are not allelomorphic to one another, but very strongly repelled. This is undoubtedly true of *B* and *I*, since they may become coupled. We may then more accurately represent the zygote $A\Theta \,.\, B\theta$ as $bc\Theta \,.\, Bc\theta$ $B\Theta \,.\, C\theta$ as $Bc\Theta \,.\, bC\theta$, and so on. Now it has been shown by Sturtevant (3) and Muller (4) that in *Drosophila melanogaster* certain factors, when present in the heterozygous condition, cause great increases in linkage, while Gowen (5) has shown that numerous factors cause small increases. If then the factors *B*, *C*, *D*, etc. have this effect in a slight degree, we should expect the linkage to be greater where the zygote is triply

TABLE II.

Female doubly heterozygous.

Mating	Number	Totals	Cross-overs
$S\theta \,.\, S\theta \times A\Theta \,.\, B\theta$	465	6	4
$C\theta \,.\, S\theta \times A\theta \,.\, B\Theta$	404, 405	72	41
$A\theta \,.\, S\theta \times A\theta \,.\, C\Theta$	192	59	32
$C\theta \,.\, S\theta \times A\theta \,.\, C\Theta$	406	26	13
$A\Theta \,.\, B\theta \times A\Theta \,.\, F\theta$	178	7	4
$BC \,.\, \Theta\theta \times A\theta \,.\, F\Theta$	235	11	2
$B\theta \,.\, P\theta \times B\Theta \,.\, C\theta$	261	24	10
$C\theta \,.\, C\theta \times B\Theta \,.\, C\theta$	347	130	60
$C\theta \,.\, F\Theta \times B\Theta \,.\, C\theta$	268	32	18
$C\theta \,.\, S\Theta \times B\Theta \,.\, C\theta$	426	5	4
$B\theta \,.\, C\theta \times B\theta \,.\, C\Theta$	232	11	8
$B\theta \,.\, Q\theta \times B\theta \,.\, C\Theta$	338	139	67
$C\theta \,.\, F\theta \times B\theta \,.\, C\Theta$	369	50	22
$C\Theta \,.\, P\theta \times B\theta \,.\, C\Theta$	398	4	1
$C\theta \,.\, P\theta \times B\theta \,.\, C\Theta$	274, 275	89	39
$J\theta \,.\, P\theta \times B\theta \,.\, C\Theta$	463	254	112
$B\theta \,.\, E\theta \times B\Theta \,.\, E\theta$	291	10	4
$BC \,.\, \Theta\theta \times B\theta \,.\, F\Theta$	257	5	1
$C\theta \,.\, F\Theta \times B\theta \,.\, F\Theta$	310, 311	47	17
$B\theta \,.\, B\theta \times B\Theta \,.\, P\theta$	197	49	21
$BC \,.\, \Theta\theta \times B\Theta \,.\, S\theta$	264	4	4
$C\theta \,.\, S\theta \times B\Theta \,.\, S\theta$	408	1	1
$B\theta \,.\, B\theta \times C\Theta \,.\, E\theta$	528	23	5
$B\theta \,.\, C\theta \times C\Theta \,.\, F\theta$	284	40	14
$B\theta \,.\, F\Theta \times C\Theta \,.\, F\theta$	312	30	18
$B\theta \,.\, C\theta \times C\theta \,.\, F\Theta$	282	40	14
$B\theta \,.\, C\theta \times C\Theta \,.\, P\theta$	285	203	88
$C\theta \,.\, C\theta \times C\Theta \,.\, P\theta$	350	72	32
$C\theta \,.\, S\theta \times C\Theta \,.\, S\theta$	413	70	35
$C\theta \,.\, C\theta \times C\theta \,.\, S\Theta$	352	40	26
$B\theta \,.\, F\Theta \times E\theta \,.\, F\Theta$	313	9	6
$C\theta \,.\, F\Theta \times F\Theta \,.\, J\theta$	389	8	3
Totals		1570	726

heterozygous, as in $Bc\Theta \,.\, bC\theta$ (or $B\Theta \,.\, C\theta$) than where it is only doubly heterozygous, as in $bc\Theta \,.\, Bc\theta$ (or $A\Theta \,.\, B\theta$).

In Table II the offspring of doubly and triply heterozygous females is tabulated. Here there were 726 cross-overs out of 1570, giving a cross-over value of 46·2 %. Where *A* was one of the factors concerned, or on the above hypothesis the females were only doubly heterozygous, there were 96 cross-overs out of 181, giving a cross-over value of $53{\cdot}0 \pm 2{\cdot}5$ % (the true value is presumably under 50 %). When *A* was not concerned, or the females were triply heterozygous, there were 630 cross-overs out of 1389, giving a cross-over value of $45{\cdot}4 \pm 9$ %. Hence in the female also linkage appears to be stronger in triple than in double heterozygotes.

These results are confirmed by an examination of the families where both parents were of the same doubly (or triply) heterozygous composition. For example the matings 175, 176, and 177 (all $A\Theta \,.\, B\theta \times A\Theta \,.\, B\theta$) gave:

116 $BB\Theta$ and $BA\Theta$, 43 $AA\Theta$, 43 $BB\theta\theta$ and $BA\theta\theta$, 2 $AA\theta\theta$,

a typical F_2 repulsion series.

The type of linkage found is similar to though far less intense than that found by Nabours (6) in *Apotettix*, where cross-over values up to 12 % were found in the female and much smaller values in the male. It is thus intermediate between the type found in *Drosophila* and *Bombyx*, where no crossing-over occurs in the digametic sex, and the type found in plants and mammals where the linkage is approximately equal in the two sexes.

Since according to Robertson (7) and Harman (8) the haploid number of chromosomes in *Paratettix* is 6 or 7, it is somewhat surprising that the loci of all the 15 factors so far discovered should be in one chromosome, which must be the case on Morgan's theory of linkage. Even if only *I*, *S*, and Θ were in different loci, the odds against finding three loci running in the same chromosome would be of the order of 48 to 1, though somewhat lessened by the unequal sizes of the chromosomes. This difficulty would be diminished if we assumed that the individual chromosomes were very rigid, but that moderate linkage occurred between chromosomes formed from adjacent parts of the spireme. This latter possibility is suggested by certain cytological observations.

The above deductions were only rendered possible by the unusual fullness of Nabours' published records, which enable the complete pedigree of any individual to be traced with the greatest ease.

SUMMARY.

Linkage between two Mendelian colour factors occurs in *Paratettix texanus*, the cross-over value being about 24 °/₀ in the male, 46 °/₀ in the female. This linkage is probably more intense in triply than in doubly heterozygous animals.

REFERENCES.

1. NABOURS, R. K. "Studies of Inheritance and Evolution in Orthoptera," I, II, III. *Journal of Genetics*, Vol. III. p. 141, Vol. VII. p. 1.
2. DEXTER, J. S. "Nabours' breeding experiments with grasshoppers." *American Naturalist*, Vol. XLVIII. p. 317.
3. STURTEVANT, A. H. "Genetic factors affecting the strength of linkage in *Drosophila*." *Proc. Am. Nat. Ac. Sci.* Vol. III. p. 555.
4. MULLER, H. J. "The Mechanism of Crossing-over." *American Naturalist*, Vol. L. p. 193.
5. GOWEN, J. W. "A Biometrical Study of crossing-over." *Genetics*, Vol. IV. p. 205.
6. NABOURS, R. K. "Parthenogenesis and crossing-over in the Grouse locust *Apotettix*." *American Naturalist*, Vol. LIII. p. 131.
7. ROBERTSON, W. R. B. "Chromosome Studies," I. *Journal of Morphology*, Vol. XXVII. No. 2.
8. HARMAN, M. T. "Spermatogenesis in *Paratettix*." *Biological Bulletin*, Vol. XXIX. No. 4.

OBSERVATIONS FAITES À VERRIÈRES PAR PHILIPPE DE VILMORIN, SUR LE CARACTÈRE "HILE NOIR" CHEZ LE POIS.

PAR A. MEUNISSIER.

PARMI les nombreuses observations faites à Verrières au point de vue génétique par le regretté Philippe de Vilmorin, et malheureusement non publiées, il n'en est pas de plus curieuses que celles ayant trait à l'étude de ce caractère.

Nous prenons la liberté de reporter ici les observations les plus intéressantes faites à ce sujet.

Dans l'immense majorité des variétés de *Pisum*, le hile est incolore et un peu plus pâle que le reste du grain. Il existe seulement quelques types chez lesquels se remarque une tache noire très nette représentant exactement le point d'attache de l'ovule développé à l'ovaire.

Nous ne savons si ce caractère existe dans les races spontanées, ni à quel moment il s'est manifesté dans les races cultivées.

Sept variétés existent à Verrières depuis plus ou moins longtemps[1].

1°. Le Pois *gris de printemps français*, variété fourragère très ancienne à petit grain vert jaunâtre et fleurs pourpres.

2°. Le Pois *Careta à œil noir* qui existe dans la collection de Verrières depuis 1877 et a été envoyé par M. Garèse d'Ovan. Le grain est rond, blanc, saumoné, les fleurs sont blanches.

3°. Un pois également à fleur blanche et grain rond, petit, blanc jaunâtre, envoyé de Chine par le R. P. Terrien en 1897.

4°. *Black-eyed Pea*, variété voisine de Careta, mais à grain plus petit. Ce pois est cultivé au Canada comme fourrage.

5°. *English Grey*, variété fourragère à fleur pourpre dont le grain est roux uni.

6°. Une variété reçue de M. Cadillan en 1904. Le grain se rapproche de celui du Careta; mais le feuillage est plus foncé.

[1] Un "Pois à cul noir" est mentionné au Catalogue Vilmorin pour l'année 1778.

7°. Un pois reçu de Chine en 1907 sous le nom de *Che-un-to* L'échantillon primitif était constitué par un mélange de grains de couleurs différentes, les uns à hile noir, les autres à hile non marqué, donnant naissance à des plantes dont les unes sont à fleurs blanches et les autres à fleurs pourpres, mais d'ailleurs identiques par tous leurs caractères de végétation.

Au point de vue des croisements, le caractère "hile noir" et le caractère "hile non marqué" constituent une paire d'allélomorphes dans laquelle "hile noir" est dominant. (Voir Tschermak.)

Cela est confirmé par les expériences suivantes:

Ridé de Knight (hile non marqué) × Careta (hile noir).

F_1 (1903) tout est à hile noir.

F_2 (1904) mélange des deux formes dans la proportion de 3 hiles noirs pour 1 hile non marqué.

Careta (hile noir) × Géant sans parchemin (hile non marqué) (1904) résultats identiques.

Clamart (hile non marqué) × Careta (hile noir) (1902) résultats identiques.

Pois à cosse violette (hile non marqué) × Careta (hile noir) 9 plantes hétérozygotes ont donné, en 1908 et 1909, 120 plantes à grain à hile noir pour 39 plantes à grain à hile non marqué soit sensiblement 3 : 1, etc. etc.

Dans ces nombreuses expériences le caractère "hile noir" se comporte comme un caractère appartenant à la plante et non au grain, absolument comme l'enveloppe du grain dans les croisements entre pois à enveloppe incolore et pois à enveloppe colorée. C'est-à-dire que la plante qui porte un des allélomorphes le manifeste sur tous les grains de toutes les cosses, et que les plantes sont, dans la proportion que nous avons étudiée, à hiles noirs et à hiles non marqués.

Dans aucun croisement nous n'avons trouvé la moindre dérogation à cette règle.

D'autre part, nous avons constaté avec étonnement que le caractère "hile noir" avait une tendance à se généraliser, c'est-à-dire à se rencontrer dans des familles pures ou des croisements n'ayant aucun lien de parenté avec les types décrits.

La première idée qui se présente à l'esprit, c'est que cette apparition de hiles noirs est due à des croisements accidentels, d'autant plus qu'ils se sont manifestés surtout depuis que les expériences faites pour étudier le caractère "hile noir" ont eu pour conséquence d'augmenter d'une façon assez considérable le nombre de plantes à hiles noirs cultivées dans la collection. Telle est, sans doute, l'origine de quelques cas.

Mais il y en a d'autres dans lesquels le caractère "hile noir" n'apparaît que sur certains grains de certaines plantes.

Ce phénomène doit être regardé d'un œil spécialement critique.

Il est des cas assez fréquents où, par suite des conditions défectueuses dans lesquelles s'opère la maturation du pois, le hile, qui est en somme un point où le grain n'est plus recouvert par son testa peut subir une modification accidentelle, être attaqué peut-être par une moisissure et paraître coloré. Nous avons assez souvent constaté cette erreur pour être en garde contre elle.

Voici quelques cas où des plantes ont présenté *des* grains et non pas *tous* les grains à hile noir.

Géant sans parchemin (*fl. pourpre*) × *Réva* (*fl. blanche*).

Dans la descendance de ce croisement dont les deux parents sont à hile non marqué, il est apparu des plantes dont tous les grains avaient le hile noir. D'autre part, en 1909, six autres lots présentaient des plantes avec grains à hiles de couleur mélangée.

1°. Sur 21 plantes, une présentait 2 grains à hile noir et une autre 5 grains à hile noir.

2°. Sur 23 plantes, deux présentaient quelques grains à hile noir.

3°. Sur 18 plantes, 17 présentaient un mélange de hiles donnant, dans l'ensemble: 164 grains à hiles noirs et 324 grains à hiles non marqués. Mais la maturation était imparfaite et le cas est douteux.

4°. Sur 13 plantes, une a tous les grains à hile noir et 6 ont des grains à hiles mélangés donnant, dans l'ensemble, 27 grains à hiles noirs et 78 grains à hiles non marqués.

5°. Sur 20 plantes, deux présentent des grains mélangés: l'une est une plante sans parchemin, conforme au reste du lot et ayant 6 grains à hiles noirs et 75 à hiles non marqués; l'autre a les cosses parcheminées et porte 19 grains à hiles noirs et 158 à hiles non marqués.

6°. Sur 19 plantes, dont 11 parcheminées et 8 sans parchemin, quatre plantes dont 2 parcheminées et 2 sans parchemin donnent des grains mélangés, soit en tout 46 grains à hiles noirs et 261 à hiles non marqués.

Pisum elatius (*fl. pourpre*) × *Mangetout nain gris* (*fl. pourpre*).

En 1909, dans un lot descendant de ce croisement, et qui n'avait jamais montré de grains à hile noir, on trouve, sur 20 plantes, deux présentant un mélange de hiles; l'une, dont les grains ont été analysés, comptait exactement 20 grains à hiles non marqués et 20 grains à hiles noirs.

La même année, et dans un autre lot issu du même croisement, 25 plantes se répartissaient de la façon suivante :

11 plantes à hiles noirs.
5 plantes à hiles non marqués.
9 plantes présentant un mélange de hiles noirs et de hiles non marqués.

Mangetout tardif à longue cosse jaune (*fl. blanche*) × *Géant sans parchemin* (*fl. pourpre*).

Ce croisement, fait en 1902 entre deux pois sans parchemin, a donné de curieuses formes et, en particulier, plusieurs lots à cosses parcheminées. C'est un de ces derniers qui a présenté, sur toutes les plantes, en 1909, une certaine proportion de grains à hiles noirs ; soit, sur 10 plantes : 185 grains à hiles noirs et 487 grains à hiles non marqués.

Pisum Jomardi (*fl. pourpre*).

Cette curieuse variété a, depuis son entrée dans la collection en 1889, donné naissance à de nombreuses formes ; probablement à la suite de croisements accidentels.

En 1908, elle a donné, pour la première fois, des grains à hiles noirs. Deux de ces grains ont été semés et ont donné, en 1909 :

1°. 95 grains non marqués et 10 grains à hiles noirs.
2°. 17 grains non marqués et 37 grains à hiles noirs.

Nous pourrions citer encore quatre ou cinq autres cas analogues.

Nous nous étendrons plus longuement sur le croisement suivant qui présente, dans sa descendance, une association bien nette et extrêmement curieuse entre les caractères "grain violet" et "hile noir" ; et qui, par suite de ce fait, a été cultivé sur une plus grande échelle.

Clamart (*grain rond, blanc et fleur blanche*) × Pisum elatius (*grain grenat et fleur pourpre*).

1903 (F_1). 6 plantes à grain roux marbré de brun.

1904 (F_2). Plantes à fleurs blanches et plantes à fleurs colorées, en proportions mendéliennes. Les plantes à fleurs blanches ont le grain blanc ; mais plusieurs plantes à fleurs blanches ont le grain blanc nettement marbré de roux.

Différents types furent suivis en F_3 et dans les générations suivantes.

En 1906 apparurent chez les plantes à fleurs colorées quelques grains violets ; et, en 1909, des grains à hiles noirs et à hiles non marqués sur

les mêmes plantes. Lorsque le coloris violet est associé au coloris "grenat," le grain paraît noir.

Le report de toutes les notes nous entraînerait beaucoup trop loin; nous résumerons simplement en un tableau les résultats observés.

Numéros de plantes	Nombre de plantes obtenues (Descendance de hiles noirs) sans grains violets	Nombre de plantes obtenues (Descendance de hiles noirs) avec grains violets	Nombre de plantes obtenues (Descendance de hiles blancs) sans grains violets	Nombre de plantes obtenues (Descendance de hiles blancs) avec grains violets	
Année 1910*					
251° *A*	—	8	—	—	
,, *B*	—	—	9	1	(à hiles mélangés)
252° *A*	2	8	—	—	
,, *B*	—	—	7	3	(à hiles blancs)
Année 1911					
523° *A*	9	8	—	—	
,, *B*	—	—	8	—	
524° *A*	1	2	—	—	
,, *B*	—	—	17	—	
525° *A*	4	5	—	—	
,, *B*	—	—	7	—	
526° *A*	—	—	6	2	(à hiles blancs)
,, *B*	—	4	—	—	
527° *A*	4	7	—	—	
,, *B*	—	—	9	1	(à hiles blancs)
531° *A*	4	7	—	—	
,, *B*	—	—	13	—	
534° *A*	4	5	—	—	
,, *B*	—	—	8	—	
535° *A*	2	7	—	—	
,, *B*	—	—	10	—	
536° *A*	1	7	—	—	
,, *B*	—	—	4	—	
537° *A*	3	6	—	—	
,, *B*	—	—	8	1	(à hiles blancs)
539° *A*	6	8	—	—	
,, *B*	—	—	6	3	(à hiles blancs et à hiles mélangés)
540° *A*	6	3	—	—	
,, *B*	—	—	15	4	(à hiles blancs et à hiles mélangés)
541° *A*	5	13	—	—	
,, *B*	—	—	17	1	(à hiles mélangés)

* Dans chaque plante semée nous avons réuni les grains en deux groupes *A* et *B*, selon la couleur de leur hile.

Nous avons omis de ce tableau les résultats donnés par les plantes dont les grains ne formaient, au point de vue hile, qu'une seule catégorie.

La coloration violette du grain est un caractère "fluctuant" que l'on n'a pu arriver à fixer. La proportion des grains violets par rapport aux grains non violets ("gris mouchetés" ou grenat) est tout à fait irrégulière et le semis des grains violets ne donne pas une proportion plus forte de grains violets; même lorsque la plante semée ne présente que des grains de cette couleur.

Numéros de plantes	Nombre de plantes obtenues (Descendance de hiles noirs) sans grains violets	avec grains violets	Nombre de plantes obtenues (Descendance de hiles blancs) sans grains violets	avec grains violets	
Année 1912					
204° *A*	5	4	—	—	
,, *B*	—	—	4	—	
205° *A*	9	2	—	—	
,, *B*	—	—	6	—	
206° *A*	5	5	—	—	
,, *B*	—	—	3	1	(à hiles blancs)
212° *A*	3	3	—	—	
,, *B*	—	—	4	—	
213° *A*	1	3	—	—	
,, *B*	—	—	3	—	
218° *A*	1	1	—	—	
,, *B*	—	—	5	—	
228° *A*	3	1	—	—	
,, *B*	—	—	5	—	
230° *A*	—	4	—	—	
,, *B*	—	—	1	1	(à hiles noirs ?)
237° *A*	2	3	—	—	
,, *B*	—	—	9	—	
272° *A*	—	1	—	—	
,, *B*	—	—	1	—	
296° *A*	2	3	—	—	
,, *B*	—	—	2	—	
298° *A*	1	2	—	—	
,, *B*	—	—	4	—	
309° *A*	4	8	—	—	
,, *B*	—	—	3	—	
325° *A*	6	2	—	—	
,, *B*	—	—	5	3	(à hiles mélangés)
326° *A*	—	7	—	—	
,, *B*	—	—	5	—	
327° *A*	3	2	—	—	
,, *B*	—	—	10	—	
330° *A*	3	4	—	—	
,, *B*	—	—	8	—	
Année 1913					
78° *A*	5	2	—	—	
,, *B*	—	—	3	—	
79° *A*	4	1	—	—	
,, *B*	—	—	8	1	(à hiles blancs)
80° *A*	1	2	—	—	
,, *B*	—	—	4	—	
83° *A*	—	1	—	—	
,, *B*	—	—	3	1	(à hiles mélangés)
84° *A*	4	1	—	—	
,, *B*	—	—	6	1	(à hiles blancs)
103° *A*	1	—	—	—	
,, *B*	—	—	4	—	

Numéros de plantes	Nombre de plantes obtenues (Descendance de hiles noirs) sans grains violets	avec grains violets	Nombre de plantes obtenues (Descendance de hiles blancs) sans grains violets	avec grains violets	
104° *A*	—	1	—	—	
„ *B*	—	—	4	—	
113° *A*	—	3	—	—	
„ *B*	—	—	2	—	
114° *A*	2	1	—	—	
„ *B*	—	—	3	—	
Année 1914					
73° *A*	—	—	9	4	(à hiles blancs et
„ *B*	1	2	—	—	hiles mélangés)
74° *A*	—	—	8	—	
„ *B*	—	4	—	—	
75° *A*	—	—	6	2	(à hiles mélangés et
„ *B*	2	8	—	—	hiles blancs)
76° *A*	—	—	43	1	(à hiles mélangés)
„ *B*	6	9	—	—	
77° *A*	—	—	14	4	(à hiles blancs)
„ *B*	6	5	—	—	
78° *A*	—	—	4	3	(à hiles blancs et
„ *B*	8	8	—	—	hiles mélangés)
79° *A*	—	—	2	—	
„ *B*	4	3	—	—	
80° *A*	—	—	21	—	
„ *B*	5	3	—	—	
82° *A*	—	—	1	2	(à hiles mélangés)
„ *B*	5	14	—	—	
83° *A*	—	—	15	1	(à hiles blancs)
„ *B*	4	11	—	—	
84° *A*	—	—	14	—	
„ *B*	7	1	—	—	
86° *A*	—	—	2	—	
„ *B*	3	13	—	—	
89° *A*	—	—	16	1	(à hiles blancs)
„ *B*	2	12	—	—	
91° *A*	—	—	4	2	(à hiles blancs et
„ *B*	1	5	—	—	hiles mélangés)
92° *A*	—	—	16	—	
„ *B*	3	10	—	—	
101° *A*	—	—	13	5	(à hiles *noirs* et hiles
„ *B*	6	14	—	—	mélangés)
102° *A*	—	—	1	—	
„ *B*	—	9	—	—	
105° *A*	—	—	3	3	(à hiles mélangés)
„ *B*	3	16	—	—	
106° *A*	—	—	17	3	(hiles ?)
„ *B*	17	72	—	—	
108° *A*	—	—	17	1	(à hiles mélangés)
„ *B*	2	4	—	—	
109° *A*	—	—	16	—	
„ *B*	2	4	—	—	
129° *A*	—	—	3	2	(à hiles blancs)
„ *B*	5	15	—	—	

Soit au total :

208 plantes. Descendance de grains à hiles noirs *ne présentant pas* de grains violets.

407 plantes. Descendance de grains à hiles noirs *présentant* des grains violets.

504 plantes. Descendance de grains à hiles blancs *ne présentant pas* de grains violets.

Et seulement :

58 plantes. Descendance de grains à hiles blancs *présentant* des grains violets.

Ou encore :

615 grains à hiles noirs ont donné *407* individus présentant des grains violets ; tandis que les *562* grains à hiles blancs, provenant des mêmes plantes, n'ont donné que *58* individus présentant des grains violets.

Dans l'état actuel de nos connaissances, ces curieux résultats sont difficilement explicables ; mais il nous a paru néanmoins qu'il pouvait être intéressant de les publier, car, comme nous l'écrivait M. le Professeur Bateson : " These exceptional negative cases may have great importance in forming a true conception of heredity."

THE INHERITANCE OF MELANISM IN THE GENUS *TEPHROSIA* (*ECTROPIS*) WITH SOME CONSIDERATION OF THE INCONSTANCY OF UNIT CHARACTERS UNDER CROSSING.

By J. W. HESLOP HARRISON, D.Sc.

(With 3 Text-figures.)

I. Introductory.

(1) *General.*

"Species are but judgments—judgments of variable value and often very fallible judgments" says Asa Gray, and certainly no genus would seem to bear more eloquent testimony to the *apparent* truth of the dictum than *Tephrosia*. Generation after generation of entomologists have used certain of its members as a kind of battleground upon which to conduct their wordy warfare as to what constitutes a species. However, it must be assumed that all of its members have formed such acute subjects for controversy; everyone, no matter whether his tendencies were to "lump" or to "split," has ever ventured to refuse specific rank to *Tephrosia luridata* (*extersaria*) and *T. consonaria*, for their position is unassailable. Far different[1] is it with *Tephrosia crepuscularia* and *T. bistortata*. Some have maintained that the two represented, at best, races of the same species, whilst others have just as stoutly insisted that they were specifically distinct. And strangely enough, although the holders of identical views did not necessarily agree amongst themselves in the reasons for the faith that was in them, each of the contending factions could only see its side of the shield, and so the battle continued. Neither side would give way, and therefore on every occasion that the question was raised it ended with matters practically in their original position.

To me the whole matter seems to hinge on the validity of Gray's definition of a species. Most of the disputants, although probably not aware that Gray had ever used the phrase, argued as if it were perfectly

[1] The same applies to certain American species of the *Tephrosia bistortata* group.

true; but, speaking for myself, and as the outcome of a prolonged study of the relationships existing between various critical species in the Lepidoptera, as well as in other groups, I am not prepared to grant its truth. Either a species is an entity or it is not; either tangible structural and other fundamental characters—physiological or otherwise—enabling one to differentiate allied forms from one another, exist or they do not. If they do not, then no amount of "judgment" would separate the forms in question so completely that one would regard them as more than local races or phases of the same species. For example, even if not so striking as those by which we distinguish *Pieris napi* and *P. brassicae*, the difference between the two Small Whites, *P. napi* and *P. rapae* is absolute; "judgment" cannot affect their status in the slightest.

(2) *The specific differentiation of* Tephrosia (Ectropis) bistortata *and* T. crepuscularia.

All of this is very pertinent to the case of *Tephrosia bistortata* and *T. crepuscularia*. Undoubtedly, to the untrained mind, they would appear to afford excellent material upon which to exercise one's judgment, so closely do they approximate in their superficial characters. The obvious differences—their depth of colour, time of emergence, their univoltine or bivoltine nature, which to the student are quite valueless because possibly (and for the most part actually) based on meteorological factors—seem to attract the casual worker like a magnet; upon them he must found his arguments. However, if one probes a little further into the matter than the mere consideration of a row or two of dried imagines in a cabinet with the data on the accompanying labels, one finds that definite structural and physiological differences manifest themselves. Although the divergences are not great, the genitalia in both sexes present significant differences, as will be seen from the figures (Figs. 1 and 2). In particular, attention is drawn to that strange and apparently meaningless dissimilarity in the knobs on the hairs of the cristae of the male apparatus (Fig. 3). Constant differences in colour and pattern, occurring throughout the common range of the two, are recognisable; the larval markings differ in design; so, too, the bright green ova of *T. crepuscularia* are always markedly distinct in cubical content and shape from the yellower ones of the related *T. bistortata*.

Furthermore, the geographical ranges of the two insects do not coincide; *T. bistortata* is Holarctic whereas *T. crepuscularia* does not

straggle beyond the Palaearctic area. Both, therefore, as we may glean, occur in Europe and Asia but, despite this, must not be regarded as having colonised precisely the same territory within these continents. *T. bistortata* is much the hardier and more adaptive insect, and therefore manages to maintain itself in much more northerly stations than its congener.

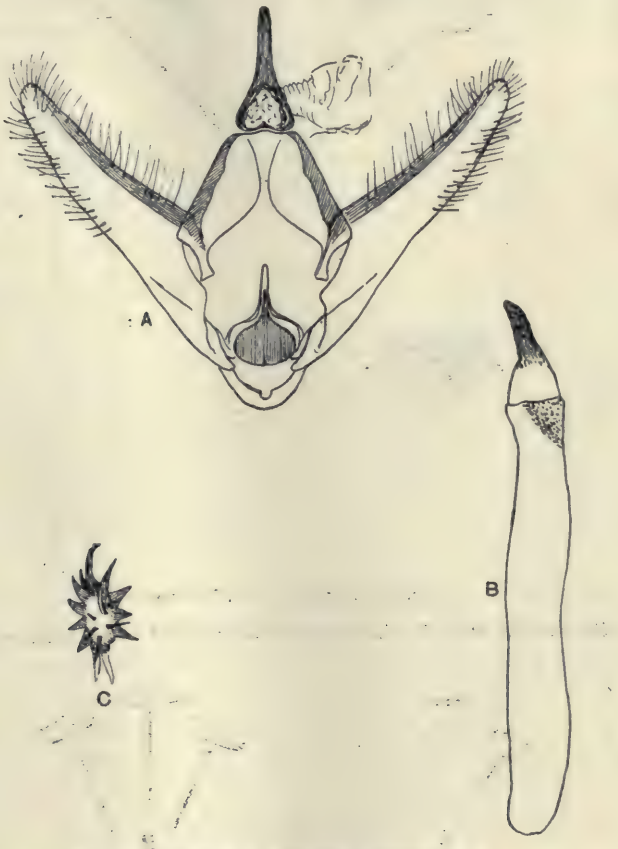


Fig. 1. Genitalia of *Tephrosia crepuscularia*.
A = Valvae, etc.; B = Ædœagus; C = Signum of female bursa copulatrix

Again, when they do occupy the same ground, they vary along different lines, and each produces its own local races. For instance, in North Durham *T. bistortata* is of the heavily barred Scottish form whilst *T. crepuscularia* is of the pale southern English type; yet in South Durham and in the adjoining Cleveland District of Yorkshire the former remains much the same whilst all the examples of the latter I have seen appertained to the melanic variety *delamerensis* B. White.

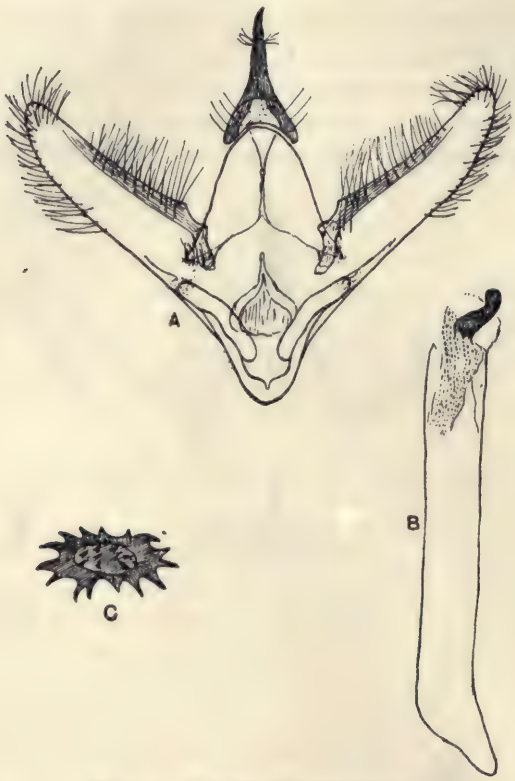


Fig. 2. Genitalia of *T. bistortata*.

A = Male valvae, etc.; B = Ædœagus; C = Signum of female bursa copulatrix.

Fig. 3. Heads of cristae hairs.

C = Cup-shaped heads of those of *T. crepuscularia*;
B = Disc-shaped heads of those of *T. bistortata*.

What is still more important, although the forms are reciprocally fertile, when the two possible crosses are made the compositions of the resulting broods are not alike. When *T. bistortata* provides the male the sexes appear in their usual proportions, but when *T. crepuscularia* supplies that sex the broods are unisexual and male under ordinary

conditions. These facts hold good no matter whence the stocks employed are derived.

Then, too, enormous displacements[1], amounting to several months, are visible in the time of emergence of these hybrid moths; and, what is still more remarkable, the acceleration displayed by the female hybrids is emphatically greater than that of the male, so that instead of the males appearing first the females do so, and may anticipate the opposite sex by several weeks.

Time, amongst other changes, brings mellowness, and now, tacitly, without the renewal of strife, but actually determined by the facts just outlined and by the process of attrition, the two have attained an impregnable position as far as their specific rank is concerned.

(3) *Melanism in the two species.*

Each species, then, as I have said, varies along its own line, but each, in spite of this, like every other species of *Tephrosia* and of the related genus *Boarmia*, produces its own peculiar melanic form or forms, that of *T. bistortata* being known as variety *passetii*, and being readily distinguishable from the corresponding form of *T. crepuscularia* to which the name *delamerensis* is customarily applied. And whenever these melanic varieties have developed in stations where the species overlap they have arisen independently and at widely different times; although Sir J. T. D. Llewelyn[2] observed the black variety of *T. bistortata* in South Wales in 1866 it was not until the early "eighties" that he captured the parallel but characteristically different melanic form of *T. crepuscularia*. Not only are these differences perceptible in the time of origin but noteworthy disagreements also exist in the headquarters of the melanic forms of each. In *T. bistortata* melanism seems to reach its greatest intensity in South Wales although I myself have captured odd specimens of melanochroic tendencies in the larch woods on Wilton Moor, 3½ miles from Middlesbrough. On the other hand, the melanic varieties of *T. crepuscularia* occur with more or less regularity in all of the great industrial areas between, and including, South Wales and South Durham.

At this point it is proper to note that, whilst undeniably displaying some degree of fluctuating variation within their own limits, of the same

[1] Such disturbances are not unusual in hybrid Lepidoptera but generally, whenever the sexes are affected unequally, except in this case, it is the male that exhibits the greater displacement.

[2] Barrett, *Entomologist's Monthly Magazine*, Vol. xxxi. p. 199.

order as that seen in the allied *Boarmia consortaria*, the melanic forms of both insects are sharply discontinuous from the types.

(4) *Object of the experiments.*

With the enumeration of these facts we are brought to the stage when the exact purpose for which the present research was undertaken may be revealed. In all of my previous work on the genera *Nyssia*, *Lycia*, *Poecilopsis*, *Ennomos*, *Drepana* and *Platysamia* it has invariably happened that, when dealing with interspecific and interracial crosses, the phenomenon of blending inheritance was encountered, and when the hybrids proved fertile there was a persistence of the blend in the F_2 and subsequent generations. On the contrary, in intraspecific crosses between aberrant or mutational forms and the type of any species ordinary Mendelian behaviour has been displayed. Naturally enough, therefore, in my mind, as in others, the thought has been engendered that the former type of inheritance might be typical of crosses between species, and that the latter was equally proper to crosses between discontinuous varieties of the same species.

In the material discussed above we have an excellent opportunity of putting these views to the test. We have two species, each with its own peculiar melanic form, which are not only fertile when cross-paired but yield progeny likewise fertile, either when mated *inter se* or when backcrossed with either parent species; and this fertility of the hybrid generations continues as long as their inbreeding is continued. By the introduction of melanism into crosses involving only one species, as well as into crosses between *Tephrosia bistortata* and *T. crepuscularia*, we can see whether its behaviour during crossing in these species supports our opinion or not. The exact question propounded for solution then becomes: Is the inheritance of melanism in both cases on typically Mendelian lines, or is it in the one case Mendelian and in the other blending?

(5) *Observations concerning the material and technique.*

Partly to render the experiment more manageable by restricting the number of broods, and partly owing to the uncertainty of securing regular supplies of ova of *T. bistortata* var. *passetii*, it was determined to introduce the melanism solely by means of the species *T. crepuscularia*, and to do this insects almost pure black in tone, save for the white subterminal band, derived from pure breeding stock, were employed. Strictly, of course, to be in accord with modern nomenclature this variety ought to be regarded as belonging to var. *nigra* Thierry-Mieg.

Practically, as the form is looked upon merely as a fluctuation of var. *delamerensis*, and would be called by that name by all British lepidopterists, and, further, as this popular conception of the insect, when viewed in the light of genetical fact is certainly sound, it will be the uniform practice throughout this paper to refer to the insect by the latter (and prior) name. Thus the use of redundant names with which modern entomological literature is so congested will be avoided.

For ova of *T. crepuscularia* type I have mainly relied on my friend Mr Goodwin of Wateringbury, Kent, save in the rare instances when I obtained typical females from Cheshire and Buckinghamshire; on the contrary, my ova of the melanic variety were derived from various sources, Birmingham, Sutton Coldfield, Delamere Forest, Wakefield, Doncaster and York, all having contributed their quota *viâ* different friends. Similarly, Mr Goodwin has always been good enough to provide me with ova of ordinary double-brooded *T. bistortata*, whilst I myself have captured the females which supplied ova of the single-brooded race, peculiar to northern and subalpine habitats, in larch woods in the Cleveland District of Yorkshire, and in North Durham.

Nevertheless, except casually, the offspring of only three melanic females has been utilised in the progress of this work. Two of these females proved, from the nature and genetical behaviour of their progeny, to have been homozygous, and the third heterozygous, for melanism.

Almost at their inception the experiments seemed doomed to failure, so difficult did it appear to rear *Tephrosia crepuscularia* of either form[1]; with exasperating uniformity the larvae either contracted some disease or the resulting pupae were few in numbers and dwarfed in size. Moreover, no amount of care and cleanliness seemed capable of removing the obstacle which at first was considered to depend on the long drawn-out larval period. Since, however, the single-brooded race of *T. bistortata* lived an equally protracted length of time in that stage and betrayed no such constitutional weakness, this possible cause was ruled out of court. Juggling with various food-plants was then attempted to neutralise the defect but *Salix caprea*, *S. aurita*, *S. fragilis*, *S. alba*, *Quercus pedunculata*, *Betula alba* and *Crataegus oxyacantha* were all quite ineffective, and even in one case, *Salix alba*, appeared to increase

[1] In view of the experience of myself and others with the superior vigour of melanic varieties of *Tephrosia consonaria*, *Boarmia consortaria*, *Oporabia dilutata*, and *O. autumnata* it is advisable to state here that, if any difference was perceptible in the two varieties of *T. crepuscularia*, the type form was the more robust; at any rate, I always seemed to rear larger broods and more vigorous imagines from type stocks.

the mortality rate. Still it was finally by manipulating the food plant that success was obtained. Once, after removing the larvae from the airtight tin boxes in which they passed their first (and occasionally their second) instars on a hawthorn diet, influenced no doubt by the incongruity of the name "*bistortata*" for an insect which prefers *Larix decidua* and *Betula alba* and also by the fact that odd larvae placed on *Cyclamen persicum* thrived amain, I placed various batches on *Polygonum*[1] *aviculare*, *Primula*[1] *acaulis* and *Primula auricula*. In all three cases the larvae did splendidly, but as *Polygonum aviculare* was the easiest plant to obtain in quantity it was subsequently always utilised in experiments with *Tephrosia*; no matter whether *T. bistortata*, *T. crepuscularia*, or any of their multitudinous hybrids, are being reared I always commence them on *Crataegus* and finish on *Polygonum aviculare*.

Further trouble developed when cross-pairing was to be attempted, as a very effective barrier to its occurrence was encountered in the difference in time of appearance of the two species; *T. bistortata* (double-brooded) emerges in March and April, whilst *T. crepuscularia* (single-brooded) is on the wing in May and June. Fortunately enough, to counteract this turned out to be an easy matter; all that proved necessary was the removal of *T. crepuscularia* pupae (wintered outside) to an unwarmed room in late February until the emergence of the first imago. As soon as that took place the *T. bistortata* cages had to be brought alongside them. Then that species commenced to come out at once since, like many other Spring Lepidoptera, they await the stimulus of rising Spring temperatures as fully formed imagines within the pupal shell for considerable periods before their escape; thus no delay is occasioned during histolysis and histogenesis.

Cross-pairing the resulting moths was an easy matter; mere confinement of the opposite sexes in small muslin cages for one evening sufficed to secure its success. However, one's difficulties did not end with successful matings. If the fertile females were not provided with roughened narrow crevices into which to thrust their ovipositors other sources of trouble cropped up. Oviposition in this pair of species apparently occurs in response to the stimulus of what Loeb would call a stereotropism. Hence, to ensure an abundance of ova, the cages must be provided with pieces of bark, crushed tissue paper, and the like. Even then failure is certain if the cages are not handled as little as

[1] The genera *Primula* and *Cyclamen* belong, of course, to the same Natural Order *Primulaceae*, and *Polygonum aviculare* and *Polygonum bistorta* are obviously both members of the order *Polygonaceae*.

possible; the females, especially those of *T. bistortata*, are extremely delicate. On the slightest provocation they develop a kind of paralysis in the legs which effectively prevents their oviposition, and speedily ends with their death. In my work all these obstacles were surmounted and fertile ova, laid in fluffy masses in various nooks and crannies, duly obtained.

II. The Inheritance of Melanism within the limits of the species *Tephrosia crepuscularia*.

To render the experiments as comprehensive as possible two sets were initiated. In both the melanism was brought into the crosses by means of a Midland insect; on the other hand, in one instance, the type insects caged up were from Kent, and in the other case extracted non-melanic moths chosen from the progeny (1) of a melanic female captured near Birmingham and (2) of a wild type female from Delamere were employed. The main purpose fulfilled in carrying out the work in this duplicate form was to determine whether the ordinary Northern and Midland form of *T. crepuscularia* which, in spite of its slightly darker and more blurred appearance, one refers to the type and assumes to be genetically equivalent to it, behaved as if it were so.

(1) *The crossing of Southern types with Northern melanics.*

From a couple of black Yorkshire females broods were reared which resembled the parents, in spite of slight fluctuating variation, very closely in colour. As they appeared they were transferred to separate cages and allowed to pair indiscriminately. Ova were obtained in due course, and from the various batches an equally random selection of two hundred ova, derived from each of the original females, was made. As before, imagines from both batches were successfully reared; since none of them showed any signs of departure beyond the limits of fluctuation of the parental and grandparental form, it was patent that both broods as well as their grandparents were homozygous for melanism, and that in each instance the original female had paired in nature with a similarly homozygous male.

Representatives of these were then mated with pale Kentish *crepuscularia*, and broods summarised in Table I bred.

Obviously, from these figures we were justified in our assumption that families *A* and *B* were homozygous for melanism, and, secondly, it is manifest that in this species melanism is dominant. However, it must be noted that some slight degree of fluctuating variation was

TABLE I.

Melanic × *Type* (*MM* × *mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
D	♀ *C* × ♂ *A**	24	17	41	—	—	—	100
E	♀ *A* × ♂ *C*	25	23	48	—	—	—	100
F	♀ *C* × ♂ *B*	18	22	40	—	—	—	100
G	♀ *B* × ♂ *C*	14	18	32	—	—	—	100
	Actually reared ...			161			0	100
	Theoretical result ...			161			0	100

* ♂ *A* simply means that the insect in question was chosen from family *A* or its inbred offspring.

visible in the F_1 broods for certain insects were paler than their melanic parent. Despite this all were undoubted melanics, and none attained, much less overstepped, the range of continuous variation amongst melanic insects taken in the woods whence the melanic stocks were secured.

These heterozygous melanic insects were paired *inter se*, back on homozygous melanics derived from a pairing between representatives of the black stocks *A* and *B*, and also with imagines bred from a fresh wild female (family *H*) from Kent. Thus the whole of the pairings possible at this stage and required in our quest were obtained with results set out in tabular form in Tables II and III.

TABLE II.

Heterozygous Melanic × *Heterozygous Melanic* (*Mm* × *Mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
I	♀ *D* × ♂ *D*	9	11	20	3	6	9	—
J	♀ *E* × ♂ *E*	13	10	23	5	3	8	—
K	♀ *F* × ♂ *F*	20	18	38	4	6	10	—
L	♀ *G* × ♂ *G*	6	8	14	2	2	4	—
M	♀ *D* × ♂ *E*	23	28	51	9	7	16	—
	Actually reared ...			146			47	75·5
	Theoretical result ...			144·75			48·25	75

This result gives us, within the limits of experimental error, the expected 3 : 1 ratio and confirms our views as to the dominance of melanism and the genetical constitution of the individuals in the F_1 generation of the cross between pure melanics and pure types.

Here again the theoretical expectation harmonises with actual fact, for the cross between homozygous melanics on the one side, and similar heterozygotes on the other, should yield only melanics.

TABLE III.

Homozygous Melanic × *Heterozygous Melanic* (*MM* × *Mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
N	♀*D* × ♂*a*	11	13	24	—	—	—	100
O	♀*a* × ♂*E*	18	23	41	—	—	—	100
P	♀*a* × ♂*E*	20	18	38	—	—	—	100
Q	♀*a* × ♂*E*	8	7	15	—	—	—	100
	Actually reared ...			118			0	100
	Theoretical result ...			118			0	100

The outcome of matings between typical homozygotes and melanic heterozygotes appears in the following table:

TABLE IV.

Heterozygous Melanic × *Homozygous Type* (*Mm* × *mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
R	♀*E* × wild ♂	14	10	24	13	8	21	—
S	♀*F* × wild ♂	6	10	16	7	6	13	—
T	wild ♀ × ♂ *F*	8	13	21	12	10	22	—
U	♀*G* × wild ♂	15	19	34	13	16	29	—
	Actually reared ...			95			85	52·8
	Theoretical result ...			90			90	50

Once more the inheritance is on lines one would have anticipated in broods proceeding from pairings between a homozygous recessive type and a heterozygous melanic when the melanism in *T. crepuscularia* behaved as a simple Mendelian dominant.

Only two possible pairings remain for treatment, and those are between homozygous black females and males of similar constitution and the parallel matings when the insects concerned are types.

TABLE V.

Homozygous Melanic × *Homozygous Melanic* (*MM* × *MM*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
A	Wild	7	11	18	—	—	—	100
B	Wild	15	16	31	—	—	—	100
A′	Ex *A*	11	14	25	—	—	—	100
B′	Ex *B*	18	20	38	—	—	—	100
a	*A′*♀ × *B′*♂	16	13	29	—	—	—	100
	Actually reared ...			141			0	100
	Theoretical result ...			141			0	100

For convenience sake in tabulation, the broods in the latter case from extracted insects and from wild stock are recorded together whilst in the former, as certain broods were lumped in obtaining the strain with which the investigations were begun, they are of necessity lumped in the table.

TABLE VI.

Homozygous Type × *Homozygous Type* (*mm* × *mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
C	Wild	—	—	—	24	19	43	0
H	Wild	—	—	—	36	32	68	0
V	Wild	—	—	—	4	6	10	0
W	Wild	—	—	—	8	7	15	0
X	Extracted *M*	—	—	—	13	9	22	0
Y	Extracted *J*	—	—	—	9	8	17	0
Z	Extracted *K* ♀ × *S* ♂	—	—	—	17	16	33	0
	Actually reared ...			0			208	0
	Theoretical result ...			0			208	0

After giving due weight to all of the observations included in the above six tables it must be concluded that, when typical insects from Kent and melanic insects from York provide the material for investigation, melanism forms a single unit character behaving in inheritance in a strictly Mendelian manner.

(2) *Similar experiments where the melanics and types were from the same or adjacent colonies.*

As the details agree so closely with those outlined above, and lead to precisely the same conclusion, the tabulated figures will be supplied with a minimum of comment.

The broods with which these experiments commenced were obtained from a wild black female from Birmingham and from a type female from Delamere Forest. As the evidence from the broods and from their subsequent behaviour proves almost conclusively that each resulted from parents one of which was a homozygous recessive and the other a heterozygous melanic, they are placed together in the table.

TABLE VII.

Heterozygous Melanic × *Homozygous Type* (*Mm* × *mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
a	Wild melanic ♀ (♂ type?)	8	10	18	7	6	13	—
b	Wild type ♀ (♂ melanic?)	13	16	29	15	13	28	—
	Actually reared			47			41	54·4
	Theoretical result ...			44			44	50

From pairings in which families *a* and *b* took part, along with others utilised in the preceding section of the work, broods were reared which gave statistics embodied in the following tables.

TABLE VIII.

Heterozygous Melanic × *Heterozygous Melanic* (*Mm* × *Mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
c	*a* ♀ × *a* ♂	2	3	5	2	0	2	—
d	*b* ♀ × *a* ♂	10	8	18	2	3	5	—
e	*b* ♀ × *b* ♂	6	7	13	2	2	4	—
f	*b* ♀ × *b* ♂	19	15	34	8	6	14	—
	Actually reared ...			70			25	73·6
	Theoretical result ...			71·25			23·75	75

TABLE IX.

Heterozygous Melanic × *Homozygous Type* (*Mm* × *mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
g	*a* ♀ × *a* ♂	6	9	15	7	6	13	—
h	*a* ♀ × *b* ♂	3	3	6	6	2	8	—
i	*b* ♀ × *b* ♂	16	13	29	17	14	31	—
j	*b* ♀ × *a* ♂	8	11	19	5	8	13	—
	Actually reared ...			69			65	51·2
	Theoretical result ...			67			67	50

TABLE X.

Homozygous Melanic × *Heterozygous Melanic* (*MM* × *Mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
k	*A′* ♀ × *a* ♂	24	17	41	—	—	—	100
l	*B′* ♀ × *b* ♂	10	13	23	—	—	—	100
m	*A′* ♀ × *a* ♂	10	8	18	—	—	—	100
n	*A′* ♀ × *b* ♂	27	19	46	—	—	—	100
o	*A′* ♀ × *b* ♂	16	18	34	—	—	—	100
	Actually reared ...			162			0	100
	Theoretical result ...			162			0	100

TABLE XI.

Homozygous Melanic × *Homozygous Type* (*MM* × *mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
s	*B′* ♀ × *b* ♂	14	18	32	—	—	—	100
t	*B′* ♀ × *b* ♂	9	8	17	—	—	—	100
u	*A′* ♀ × *b* ♂	21	24	45	—	—	—	100
v	*b* ♀ × *B′* ♂	8	3	11	—	—	—	100
	Actually reared ...			105			0	100
	Theoretical result ...			105			0	100

TABLE XII.

Homozygous Type × *Homozygous Type* (*mm* × *mm*).

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
p	*a* ♀ × *a* ♂	—	—	—	14	12	26	—
q	*b* ♀ × *b* ♂	—	—	—	29	26	55	—
r	*a* ♀ × *b* ♂	—	—	—	25	18	43	—
	Actually reared ...			0			124	0
	Theoretical result ...			0			124	0

The result of mating homozygous melanic insects together may be seen by referring to Table V above, where it will be perceived that as the outcome of such pairings nothing save melanic insects appeared.

Thus it seems that, no matter whether the type insects are obtained from localities totally uncontaminated with melanism or from stations where that is rampant, the result is the same; melanism in the species *Tephrosia crepuscularia* is inherited strictly in accordance with Mendelian expectation. From this we may draw the conclusion that, as far as that character is concerned, typical insects from all sources are genotypically the same.

III. The Inheritance of Melanism introduced by *Tephrosia crepuscularia* var. *delamerensis* when that species is crossed with *T. bistortata*.

As the source of the *Tephrosia bistortata* utilised in the experiments I selected a Wateringbury brood (designated hereafter as Family I) reared from a wild female. Needless to say this brood[1] was, as far as melanism was concerned, composed of homozygous recessives. Reciprocal crosses were made between insects of this origin and *T. crepuscularia* of the *delamerensis* form chosen from the two broods labelled *B′* and *a* above. As will be remembered, *B′* included only homozygous melanics whereas "*a*" provided homozygous types as well as heterozygous melanics.

The following tables give concisely, as far as colour and sex are concerned, the indications of the original hybridisation experiments. Except for the abnormal sex results set out in Table XIV A, which form the subject of a subsequent paper, they call for little comment. They are in practical agreement with Mendelian requirement although perhaps the proportion

[1] Experimental evidence exists to the effect that in *T. bistortata* as in *T. crepuscularia* melanism is dominant.

TABLE XIII.

Homozygous Type bistortata ♀ × *Homozygous Melanic* crepuscularia ♂.

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
II	I ♀ × B' ♂	56	11	67	—	—	—	100
III	I ♀ × B' ♂	45	0	45	—	—	—	100
IV	I ♀ × B' ♂	61	0	61	—	—	—	100
Actual result[1]				173			0	100
Expectation if inheritance occurred on a Mendelian basis ...				173			0	100

TABLE XIV A.

Homozygous Type bistortata ♀ × *Heterozygous Melanic* crepuscularia ♂.

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
V	I ♀ × b ♂	22	—	22	30	—	30	—
VI	I ♀ × b ♂	36	—	36	37	—	37	—
Actual result...				58			67	46·4
Expectation if inheritance occurred on a Mendelian basis ...				62·5			62·5	50

TABLE XIV B.

Heterozygous Melanic crepuscularia ♀ × *Homozygous Type* bistortata ♂.

Family	Parentage	Melanics			Types			Percentage Melanic
		Males	Females	Totals	Males	Females	Totals	
VII	a ♀ × I ♂	18	12	30	9	36	45	—
VIII	a ♀ × I ♂	26	17	43	28	37	65	—
Actual result...				73			110	39·7
Expectation if inheritance took place on a Mendelian basis				91·5			91·5	50

of typical insects in the last two broods[2] is somewhat high. Curiously enough, too, the percentage of types in both cases is exactly 60%. However, this excess is in all probability correlated with the slightly greater constitutional vigour possessed by the type forms tending to their preferential survival.

The matter of the inverse relationship between sex and colour may be connected to some extent with the same phenomenon, but, in face of the corroborative evidence supplied by the two broods, not merely

[1] I have had precisely the same result with two large broods of this hybrid reared during March and April 1920 for another purpose.

[2] The sex ratio (102 ♀ ♀ : 81 ♂ ♂) in the case of these broods is not, I think, significant. In a parallel cross between type *crepuscularia* ♀ and type *bistortata* ♂ I obtained 96 ♀ ♀ and 88 ♂ ♂.

by the existence of the fact but by the approximation of the percentages of each sex and colour they display, the matter must be submitted to further investigation.

Returning now to the question of the inheritance of the melanism, since the hybrids were perfectly fertile, matings were made between homozygous type males from brood VII and females of the same source and form, as well as between heterozygous melanics chosen from the same batch. Furthermore, males from family III and, in one case, a wild second brood male *bistortata* were caged up with typical females from brood VII. Just as in the case of homozygous recessives extracted in the course of the experiments detailed in Section II nothing but types (16 ♀ ♀ and 34 ♂ ♂) were obtained, and the same holds good with the back cross with *T. bistortata* where the results are 32 ♀ ♀ and 33 ♂ ♂. Here again we see nothing antagonistic to Mendelian anticipations.

Widely opposed to this was the evidence submitted by the other two crosses. In the second cross (family IX), where F_1 heterozygous melanic insects were mated together, so chaotic were the F_2 imagines (22 ♀ ♀ and 39 ♂ ♂) in appearance, and so wide was their range of variation that neither description (except as individuals!) nor classification of the imagines seems possible. In addition to this, an F_3 generation bred from these nondescripts simply reproduced the parental condition and equally defied classification. Strangely enough, careful study of the exact tendencies of these broods suggests that they lean most strongly to paleness; captured wild amongst first brood *T. bistortata* very little attention would be attracted by their patterns.

Nor are matters greatly improved with the other cross (family X) of parentage (*crepuscularia* ♀ × *bistortata* ♂) ♀ × (*bistortata* ♀ × *crepuscularia delamerensis* ♂) ♂ which gave rise to 21 ♀ ♀ and 57 ♂ ♂. Of these the following is an attempted classification on a colour basis:

(*a*) One male was as dark as its male parent.

(*b*) Eight (all males), varying amongst themselves, whilst not black, are dark enough to excite little or no comment.

(*c*) Two (both females) were palish, but nevertheless powdered with dark scales.

(*d*) Thirty-seven (four males and thirty-three females) were so variable and transitional as to baffle description. As far as their general coloration is concerned, however, none transgress beyond the range of variation shown by North Kent *bistortata*; they are simply separated from section (*e*) because of their variation in other respects.

(*e*) Thirty (sixteen females and fourteen males) would be correctly described as pale, although all were sprinkled more or less with fine blackish powdering. One alone perhaps if inserted in a series of pure *T. crepuscularia* would have passed unchallenged.

Despite this attempt to disentangle their variation, from this family, as from the last, we gain the impression which entirely negatives any occurrence of segregation on Mendelian lines in the gametogenesis of both sets of F_1 hybrids. A full examination of the possible causes of this failure and of other results of these experiments will be found in the general discussion below.

Before this is undertaken one further and extremely interesting fact must be chronicled. In families VII and X, and in one[1] obtained from a crossing between ordinary types of *T. bistortata* and *T. crepuscularia*, parthenogenesis was observed in the case of four females, one from each of the first two broods and two from the last named. Of these only the first put us in possession of facts vital to the present research, although only three imagines (one female and two males) were reared.

The female would not be out of place either in brood IX or in the nondescript portion of family X. On the contrary, one of the males resembles the blackest forms bred, whilst the second is a beautiful insect of form not previously encountered. Probably it ought to be described as pale, as its ground colour is white, heavily "peppered," and shaded with dove grey scales, the shadings becoming more intense terminally. On both anterior and posterior wings it exhibits a strengthening of the median black line, but all of the other transverse lines, in addition to the usual white subterminal band or line, are obsolete. It strongly reminds one of some of the caesious species of the genus *Gnophos* in all of its characteristics.

IV. Discussion of the Results.

We have discovered a case in which one and the same unit character displays inheritance in absolute conformity with Mendelian conceptions at one time, yet at another behaves so aberrantly as to suggest the workings of a scheme of inheritance fundamentally different from the former, the character in question being introduced by insects from the same brood. Let us, however, be careful to explain that the phe-

[1] And later in a female of parentage (*crepuscularia* ♀ × *bistortata* ♂) ♀ × (*bistortata* ♀ × *crepuscularia* ♂) ♂.

nomena observed in the last-named experiments were not those of ordinary blending inheritance. Not only was there apparent dilution to varying extents of a proved unit character, but in addition the fluctuations obtained were so chaotic and irregular as to indicate the action of some disturbing influence dislocating to a profound extent the mechanism of Mendelian heredity as well as of that permitting a blend. In the original set of experiments we were dealing with crosses between different varieties of the same species, whereas in the latter the crossings were interspecific; for the inciting agents in the disturbance, therefore, we have to look to circumstances arising out of the specific distinctness of the insects used in the second series of experiments.

Although not concerned with melanism, many other workers, when working with species- and race-crosses, have met with irregularities more or less parallel with mine. For instance, both Bonhote and the two Hagedoorns, describing their hybridisation experiments with *Mus rattus*, *M. alexandrinus*, and *M. tectorum*, mention rats (waltzers and the like), departing widely from the normal, as appearing in their F_2 cultures. Neither of these two investigators are prepared to grant that their animals are mutants in the genetical sense of the word. Although their views are possibly correct, for my part I feel sure that, no matter how induced, their origin, as well as that of my aberrant moths, must be assigned to causes precisely the same as those giving rise to mutations. From this it seems hardly necessary to state that from their further explanations I emphatically dissent. In the opinion of the Hagedoorns we have to look to the joint action of two recessive[1] characters whose cooperation was impossible until the chance rearrangement of factors brought about by segregation in F_1 gametogenesis and their union in the F_2 zygotes ensured it.

Bonhote, on the other hand, perceives the origin of the new characters in a new environmental complex diminishing the initial vigour of the animals he used.

Not essentially different from these results, and perhaps not so striking although more in harmony with mine, are those of Sumner, who worked with local races of the Californian Deermouse (*Peromyscus maniculatus*). In the F_2 generation of hybrids between *Peromyscus maniculatus sonoriensis* and *P. maniculatus rubidus* he obtained what he terms "partial" albinos; these he regards as almost certainly true mutants, whose inception must be ascribed to the loss of a gene during

[1] I might agree with this if their "recessive" characters be regarded as equivalent to "absences"!

the hybridisation of such diverse strains. Incidentally, in expressing this opinion, he refers to the Hagedoorns' work, and makes the definite assertion that one must not assume that their novelties would always be generated by crossing animals of the same racial composition. Had the observed results been confined solely to the Hagedoorns' experiments in America, such a criticism perhaps would have been completely justified, but when we recognise that the same types of animals were obtained quite independently by Bonhote in England, in hybrids of like composition, the conclusion is irresistible that the same disturbing cause has sufficed in two perfectly distinct instances to produce the same effect.

Combined with mine, the evidence yielded by all these crosses tends to demonstrate that in interspecific and interracial hybrids novel forms, differing it is true in degree, but nevertheless quite new, are liable to develop. Moreover, judging from their unusual nature, they are not the products of ordinary gametic segregation and recombination; their exact origin therefore remains to be discovered.

Most geneticists, although not assenting *in toto* to Morgan's statement that now "the problem of heredity has been solved"[1], seem in substantial agreement that it is the chromosome complex upon which the actual mechanism of heredity and development depends. Admitting the validity of this view, it appears certain that should any alteration take place in the normal chromosomal distribution, either by the irregular removal or displacement of the part or whole of any chromosome or chromosomes, such a dislocation must tend to the origination of organisms of an aberrant type. And, fortunately enough, cytological investigation has revealed the presence of such abnormalities of chromosome behaviour in the gametogenesis of interspecific and interracial hybrids. In particular they have repeatedly been detected by Federley, Doncaster, and myself in hybrid Lepidoptera.

Amongst the insects in which I have observed the occurrence[2] are the very Tephrosias we are discussing. In the gametogenesis of their hybrids many vagaries in behaviour are encountered; often enough, in the normal pairing of the chromosomes in preparation for the maturation divisions, many chromosomes fail to find mates, so that in the subsequent separation, although the individual members of those paired correctly proceed as usual to the opposite poles of the spindle, in other

[1] *I.e.* by granting that the material basis of heredity is located in the chromosomes.

[2] The finest examples I have seen of such mitotic dislocations were in certain hybrids of the genus *Rosa*.

cases whole chromosomes are lost in the cytoplasm, others pass undivided to one pole, others divide regularly or irregularly, and so on. Thus the gametes finally developed are endowed with varying chromosome complements. Clearly enough the gametes produced by the fusion of such gametes will also possess abnormal chromosome combinations, so that on the completion of development the insects bred ought, as just urged, to be of novel guise.

How does that help to explain the present series of facts? If in the gametogenesis of the F_1 *bistortata-crepuscularia* hybrids the chromosome carrying the gene for melanism finds its homologue, and this pair of chromosomes follow the usual course of division with genes uncontaminated, or should it in default of finding a mate pass undivided to one pole, then in either case ordinary Mendelian ratios should obtain amongst the F_1 insects. Such ratios did not manifest themselves, so we are driven to consider further explanations.

Giving due weight to the cytological facts, the following possibilities lie open:

(1) that the chromosome in which the factor for melanism lies is amongst the number of those which, during the first maturation division, split equationally, which in turn demands the probability that the chromosome homologous to it should do likewise;

(2) that it divides into unequal portions;

(3) that the homologous chromosomes pair as in the gametogenesis of a pure species, but that whilst they are united a levelling up or averaging of the values of their component determiners ensues; this would necessitate, on separation, the production of hosts of gametes with varying powers so far as the induction of melanism is concerned, their variation in this respect depending on the degree of completeness of the blend;

(4) that the chromosome carrying the gene for melanism is lost in the cytoplasm during the later stages of division.

Distinct as they would seem to the first glance, possibilities (1) and (2) are not different in effect unless one is prepared to grant that a mere mechanical division is able to fractionise the determiner for a given Mendelian character. If it is not possible so to break it into smaller fractions then, since it must proceed during cell division one of two ways unaltered, no departure from Mendelian expectation should result. For these reasons this possibility must be dismissed. In any case, even if it were possible to split a gene, then the resulting broods should have

been predominatingly melanic, which is diametrically opposed to their actual condition.

Similarly the fourth supposition, although almost certainly the correct explanation of the more extraordinary of the Hagedoorns' and Bonhote's rats, appears in itself inadequate to account for every peculiarity of my F_2 and F_3 broods, although doubtless to its action one must assign the overweighting of the paler classes and the weird designs of some of the insects. Therefore we must fall back on the third consideration to account for the bulk of the facts. In my opinion, this contamination of the gametes, or, to put it bluntly, the inconstancy during crossing of a character demonstrated by other and intraspecific experiments to behave as a unit character, operating in conjunction with the circumstances referred to in (4), affords the only satisfactory interpretation of the experimental data. To the same effect, and powerfully supporting the views just enunciated, is the evidence supplied by the imperfect segregation observed in the imagines developed parthenogenetically from the heterozygous F_1 melanic female. One warning must be given; all this necessarily demands that in species- and race-hybrids this gametic blending or contamination is more prone to occur than in crosses between varieties of the same species. That being so, the occurrence must be intimately connected with the inability of the chromosomes to play their normal parts owing to partial or total incompatibility.

Now if changes in gametic values such as described be deemed proved—and I see no other possible explanation when due regard is given to the perfect Mendelian behaviour of the melanism in the mating of *T. crepuscularia* with its black form *delamerensis*—one must be prepared to admit the truth of Castle's contention, backed up by the evidence provided by his researches into the genetics of hooded rats and English rabbits, that unit characters are inconstant and therefore capable of response to selection. Even if they are but slight, physiological divergencies of low grade, but of the same type as those distinctive of recognised species and races, must exist between individuals of the same species. No matter how unimportant, if of the same order, these differences must have similar effects, and thus determine a change in what is customarily reckoned a unit character, just as Castle insisted.

At any rate, granting that a definite change in a unit character, melanism to wit, has not been proved absolutely within the bounds of a species, it must, nevertheless, be admitted that conclusive proofs have been supplied that the given character has been modified during an interspecific cross. If this is so, it seems a very minor extension of our

deductions from the experimental evidence to assert that changes in the same direction are assuredly possible at other times no matter how induced, be it by crossing or otherwise.

Had the indications of my *bistortata-crepuscularia* hybrids stood alone, to the Neo-Mendelian they would simply have afforded a further illustration of the truth of Nilsson-Ehle's hypothesis of cumulative factors. He would have triumphantly pointed out the uniformity of the F_1 lots and the great spread of variation in the F_2 imagines and heralded them as proof positive of his theory, so beautifully do they harmonise with it. To him the sole import of the experiment would have been to demonstrate that melanism, far from depending on a single factor, arises through the cumulative action of many.

However, its behaviour in the intraspecific crosses confutes this emphatically; then it comports itself without any shadow of doubt as a unit character. This effectively removes the power from the same far-fetched (although logical from the Neo-Mendelian standpoint) counter-argument considered so destructive in the analogous case of Castle's rats. Hoodedness, we were informed, in that case simulated a unit character merely because coexistent with it was a definite "character" without which its manifestation was impossible. Its appearance thus being interdependent on the effect of this single suppositious "prompter," its behaviour must be that of a unit character. Once it was permitted to appear the play of varying numbers of multiple factors would admit of variation in the degree of hoodedness, and therefore render possible selection in respect to the character.

No such "prompter" can be dragged in to entangle my results in the web of the multiple factor theory. The disparity between the enormous fluctuation of the melanism, even to its actual disappearance, in the F_2 *crepuscularia-bistortata* hybrids, and the uniformity of the melanics in the F_2 *crepuscularia-delamerensis* mongrels is too striking to allow it. The only feasible scheme capable of accounting for the facts is to declare that species-hybridisation has modified permanently a unit factor for melanism and has very possibly been responsible in certain cases for its loss.

Simply invaluable in furnishing further proof of this is the condition of the insects bred parthenogenetically from the F_1 *crepuscularia-bistortata* melanic female. Here one of the insects transgressed the degree of melanism of its parents (and even its blackish grandparent). Had this happened with zygotes produced by normal fertilisation, its existence would have unhesitatingly been assigned to the presence of

factors for melanism in both of the original parents which were piled up in the F_2 insects to an extent quite impossible in either of the pure strains or in the F_1 broods.

As, however (even were the truth of that hypothesis admitted), the parthenogenetic male could only at the most possess the same number of such factors as its parent, and very possibly actually less, the multiple factor theory of transgression in F_2 broods is not supported. With the removal of this further prop, coupled with the destructive evidence offered by the dissimilar results of the two sets of work just described, it seems to me that a very shrewd blow has been dealt to the applicability of the hypothesis to the critical case of Castle's rats.

Let us now examine the matter from a somewhat different angle. Opinion[1] has been settling to the view that the differences between species are, at bottom, chemical, and that diverse cytoplasms reacting and interacting with diverse enzymes located in the chromatin of the cell nucleus are responsible for opposed, specific characters. Apply this concept to the present problem, and one readily perceives that the introduction of enzymes foreign to the cytoplasm of an egg might interfere very profoundly with the regular course of development; in our case the intensity of the melanism might be so influenced as to suggest a very significant departure from the usual Mendelian anticipation. Two very forceful arguments serve to destroy this idea. Firstly, the disturbance (if any), if due to the difference in interacting chemical substances and to no other circumstances whatever, should be at its maximum in the F_1 generation which, as we know, is not correct. And secondly, since the amounts of cytoplasm supplied by ovum and spermatozoon are so unequal, if there were any truth in it, obvious differences should exist between the results of reciprocal crosses between the two species. This is not so for the two lots of insects are substantially alike; whether the melanism is brought in by a *crepuscularia* male or female the broods agree inasmuch as the melanism acts in the F_1 lots as a simple Mendelian dominant. Only in F_2 insects and those of similar origin do the abnormalities make their presence felt, and this at a time when one would have anticipated that cytoplasmic values, chemically speaking, would have attained their mean.

From this position only one logical path of escape lies open, and that is to admit that during the progress of hybrid development the enzyme responsible for melanism, whether by stereoisomerism and the

[1] Reichart has admirably summarised the facts concerning the chemical distinctness of species, etc. in *Science*, Nov. 6, 1914.

like, or by some trivial alterations in the constitution of its sidechains, has been so changed as to initiate different reactions in the building up of the F_2 insects—a conclusion differing in terms but essentially the same in effect as that deduced from other considerations. Again it simply amounts to stating that, in crosses, the genes or determiners for unit characters are liable to undergo modification.

To sum up, the results of our research are as follows:

(1) In crosses within the limits of the species *Tephrosia crepuscularia* the inheritance of melanism proceeds on ordinary Mendelian lines; this is in complete agreement with the work of Onslow and myself in *Tephrosia consonaria* and *Boarmia consortaria*.

(2) This character in interspecific crosses between *Tephrosia crepuscularia* and *T. bistortata* behaves in a vastly different fashion.

(3) From the indications of the last set of experiments one can only conclude that unit characters may be changed during such crosses.

(4) If the last statement be true (and there appears no alternative to admitting its truth), then the possibility of modifying such characters by other agents cannot be excluded.

LITERATURE CITED.

Bonhote, J. L. *Vigour and Heredity.* London. (1915.)

Castle, W. E. "The Inconstancy of Unit-characters." *American Naturalist*, Vol. xlvi. pp. 352—362. (1912.)

—— and Hadley, P. B. "The English Rabbit and the Question of Mendelian Unit-character Constancy." *Proc. Nat. Acad. Sci.* Vol. i. pp. 39—43. (1915.)

Federley, H. "Chromosomenstudien an Mischlingen. I. Die Chromosomenkonjugation bei der Gametogenese von *Smerinthus populi* var. *austauti* × *populi*." *Öfversigt af Finska Vet. Soc. Förh.* Bd. lvi. A. No. 13. pp. 1—28. (1914.)

Hagedoorn, A. C. and A. L. "Rats and Evolution." *American Naturalist*, Vol. li. pp. 385—418. (1917.)

Harrison, J. W. H. "Genetical Studies on the Moths of the Geometrid Genus *Oporabia*." *Journ. Genet.* Vol. ix. (1920.)

—— and Doncaster, L. "On Hybrids between Moths of the Geometrid Subfamily *Bistoninae*." *Journ. Genet.* Vol. iii. pp. 234—248. (1914.)

Loeb, J. *Forced Movements, Tropisms and Animal Conduct.* Philadelphia and London. (1918.)

Morgan, T. H. *Critique of the theory of Evolution.* Princeton. (1916.)

Nilsson-Ehle, H. "Einige Ergebnisse von Kreuzunger bei Hafer und Weizen." *Bot. Notiser*, pp. 257—294. (1908.)

NILSSON-EHLE, H. "Kreuzungsuntersuchungen an Hafer und Weizen." *Lunds Univ. Årsskr.* N. S. II. 5, pp. 1—122. (1909.)

ONSLOW, H. "Inheritance of Wing Colour in Lepidoptera. II. Melanism in *Tephrosia consonaria* (var. *nigra* Banks)." *Journ. Genet.* Vol. IX. pp. 53—60. (1919.)

—— "III. Melanism in *Boarmia consortaria*." *Journ. Genet.* Vol. X. (In the Press.)

SUMNER, F. B. "Continuous and Discontinuous Variations and their Inheritance in *Peromyscus*." *American Naturalist*, Vol. LII. pp. 177—208, 290—301, 439—454. (1918.)

—— "Several Color 'Mutations' in Mice of the Genus *Peromyscus*." *Genetics*, Vol. 2, pp. 291—300. (1917.)

ON THE LEFT-SIDED INCIDENCE OF THE SUPERNUMERARY DIGIT IN HETERODACTYLOUS FOWLS.

By C. J. BOND, C.M.G., F.R.C.S.

THE following results have been obtained by crossing representatives of certain five-toed with four-toed breeds of Fowls.

The breeds chosen were the Silky, Dorking and Faverolle on the one hand and the Old English Game, Wyandotte and Orpington on the other. The proportion of four-toed to five-toed and heterodactylous offspring (birds with five toes on one foot and four on the other) varied somewhat according as the male or female parent belonged to the five-toed breed and also in the F_1 as compared with the F_2 generation.

Out of a total however of 402 birds 172 developed four toes on both legs, 192 five toes on both legs and 38 were heterodactylous.

Of these heterodactylous birds 34 had the extra digit on the left side and only 4 on the right side.

The proportion between the numbers of four-toed, five-toed, and heterodactylous birds in the different matings and in the two generations is shown in the following table:

Mating	Generation	Four-toed	Five-toed	Heterodactylous
G. cock × Silky hens	F_2	8	5	2
Silky cock × Game hens ...	F_2	17	14	4
S. Dorking cock × Orpington hens	F_1	2	35	4
,, × ,,	F_1	16	22	4
ditto (*a*) mating	F_2	15	17	1
,, (*b*) ,,	F_2	18	39	7
,, (*c*) ,,	F_2	30	18	5
,, (*d*) ,,	F_2	17	18	4
Cross back to parent 4-toed stock	F_2	29	12	2
Faverolle cock × Orpington hens...	F_1	8	7	0
,, × ,,	F_2	12	5	5
Totals		172	192	38

The tendency of the Extra Digit to appear on the Left Side in the Asymmetrical Birds.

In the early matings the supernumerary digit appeared on the left side in all the asymmetrical birds and it was only on mating a heterodactylous cock with heterodactylous hens of the F_2 generation that the extra

digit appeared on the right side in two cases. Two more examples occurred in a mating between a heterodactylous cock and five-toed hens.

The tendency for the fifth digit to appear on the left side is evidently strong in birds which are heterozygous as regards number of digits.

Besides these heterodactylous birds a number of the symmetrical five-toed hybrids also showed a fuller development of the fifth digit on the left side. In some cases this left-sided extra digit was made up of a larger number of phalanges, and in some these phalanges were better developed. In two birds rudiments of six toes appeared, in one case on the left and in one on the right side.

This over-development in excess of the number of digits normally possessed by a five-toed breed is important and suggests that the fifth toe in the five-toed race is not transmitted to hybrid offspring as a stable unit character, but rather that the factor transmitted when a five-toed is crossed with a four-toed breed is a tendency to extra serial segmentation of the rudiments forming the digits and not a tendency to develop a fixed number of (five) digits.

This serial segmentation may affect the whole digit or the phalanges only, it may be complete or incomplete and in the latter case a fused metatarsal bone or phalanx may be common to two digits.

Although the numbers in which the reverse cross was tested are small they suggest that heterodactylism with the extra digit on the left side is more frequent when the female parent belongs to the five-toed breed.

This left-sided incidence of the extra toe in asymmetrical birds is confirmed by other workers. Thus Messrs Bateson and Punnett have kindly furnished me with the following records of extra toed chicks bred by them. In 89 asymmetric birds from different matings the extra digit appeared on the left side in 72 cases and on the right side in 17. In 49 birds the extra digit although present on both sides was unequally developed, the more fully developed toe occurred on the left side in 34 cases and on the right side in 15.

Since this paper was written Prof. Punnett has also called my attention to articles by D. Barfurth in the *Archiv für Entwicklungsmech. der Organismen*, XXXI and XXXIII. In a summary of different matings between normal and hyper-dactylous fowls carried out between 1908 and 1910 Barfurth gives a total of 1031 individuals of which 556 were four-toed and 475 hyper-dactylous. Of the hyper-dactylous birds 410 were symmetrical and 65 asymmetrical. Of the 65 asymmetrical birds the extra digit was present on the left side in 35 and on the right side

in 30 cases. In a later table (*ibid.* XXXIII) Barfurth tabulates 40 hyper-dactylous birds of which 8 were asymmetrical and of these 6 were left-sided and 2 were right-sided. In a final list of 18 asymmetrical birds 12 were left-sided and 6 right-sided.

Barfurth also records in the same journal some observations on extra rudimentary digits in the wing bones of chicks. Of 21 individuals so characterised 2 were asymmetrical, the extra rudimentary digit in both cases affected the right wing. In another group of 79 birds 6 asymmetrical cases occurred all with the extra digit on the left side.

It is of interest to compare this asymmetry of toe development in heterozygous fowls with other characters which under normal conditions are symmetrically distributed.

In *Polydactylous Guinea Pigs* there is a slight but definite tendency for the supernumerary digit to appear on the left side, thus Castle (*The Origin of a Polydactylous Race of Guinea Pigs*, Pub. Carnegie Instit., May 1906) states that out of a total of 1219 individuals the extra toe appeared on the left side in 630 and on the right side in 582. Attempts to increase this asymmetry however failed.

In an unpublished enquiry into the prevalence of *Supernumerary Nipples in Guinea Pigs*, Mr Pechey of Leicester finds that out of 56 individual offspring of parents with supernumerary nipples 17 possessed supernumerary nipples or teats. Of these 17, 10 were asymmetrical, the extra nipple being placed on the left side in 7 cases and on the right side in 3 cases. In 6 individuals with extra nipples on both sides the larger and more fully developed teat was situated on the left side. One individual (a female) had four extra nipples symmetrically placed. These records show that a tendency exists for the supernumerary nipple to appear on the left side in guinea pigs.

Asymmetric Tail Feather Pattern in Pigeons.

A blue-barred Homer cock with the normal number (12) of tail feathers was mated with an inbred Fantail hen having 25 tail feathers. From this Homer-Fantail cross 21 individual birds of the F_1 generation gave 140 tail feathers on the left side and 140 on the right.

In 50 F_2 birds from the same cross there were 902 tail feathers, 456 on the left side and 446 on the right.

Although the distribution of fully developed tail feathers in these hybrid Fantail birds only shows a slight preponderance of numbers on the left side in the F_2 generation, further evidence of asymmetry is

obtained in the distribution of certain abnormal double tail feathers which occur in these Fantail hybrids. Morgan has drawn attention to the occurrence of these double tail feathers in cross-bred pigeons but does not mention any inequality of distribution.

In the above cross these abnormal tail feathers appeared in 12 cases in the F_1 offspring, 5 being on the left and 7 on the right side. In the F_2 generation however out of 11 examples 8 occurred on the left side and only 3 on the right.

In a total of 38 abnormal tail feathers from this and other matings 21 occurred on the left side and 17 on the right.

Evidently the condition of unstable equilibrium in the number and distribution of tail feathers in hybrid pigeons, the result of crossing a fish-tailed with a fan-tailed breed occurs on both sides of the body, but the numbers suggest a slight excess of meristic variation away from the normal 12 feather arrangement on the left as compared with the right side of the body.

Taking the facts concerning heterodactylism in fowls and tail-feather pattern in hybrid pigeons together we find that (in birds at any rate) there is a tendency during the early cleavage of the ovum which separates the organism into a right and left half, for the factor or factors which control meristic variation to pass to the left rather than to the right half of the developing zygote.

In this article it is not intended to discuss the question whether any association exists between the left-sided incidence of asymmetrical characters and sex. The atrophy of the right ovary in normal female birds is important in this connection, so also is the fact that in a certain number of female heterodactylous fowls the extra digit on the left side is associated with the development of a spur on the same side. Heinrich Poll (*SB. Ges. Naturf. Fr. Berlin*, 1909) states that in true hermaphrodite birds the male secondary sex character tends to appear on the right side, that is the side on which the primary sex gland is male in these abnormal birds. There are difficulties in accepting the hormonic theory as a full and complete explanation of the occurrence and distribution of secondary sex characters in birds. The case of the hermaphrodite pheasant with hemilateral asymmetry of secondary sex characters recorded in the *Journ. Gen.*, Feb. 1914, together with other examples recorded by other observers suggests that (in birds at any rate) in addition to the influence exercised by sex hormones on the *development* of secondary sex characters other factors are concerned in their *origin and distribution*. One other such factor may be a capacity

on the part of the body cells of the zygote to respond in a different way to the hormonic substances formed by the female and male primary sex glands respectively. In normal individuals this capacity of tissue response is distributed symmetrically on both sides of the body, whereas in these abnormal hermaphrodite birds it would seem to be located asymmetrically, and it is of interest to find that other examples of hemilateral asymmetry such as the extra digit in heterodactylous fowls also occur in birds.

VOLUME X AUGUST, 1920 No. 2

INHERITANCE IN BARLEY.

I. THE LATERAL FLORETS AND THE RACHILLA.

BY F. L. ENGLEDOW.

(With 3 Text-figures.)

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INTRODUCTION.

THIS account of the inheritance of certain of the characters of barley is to be regarded as provisional. It represents the results of observations made from time to time upon F_2's which have been raised for plant breeding purposes.

The "lateral floret" has become a familiar problem in barley genetics. It is believed that although the general solution of this problem has been reached, there still remain a number of interesting side issues which can be decided only by raising large and complete F_3's and by a histological study of the various degrees of development of the floret.

That the artificially produced variety *H. inerme* (obtained by Rimpau from the cross *H. Steudelii* × *H. trifurcatum*) offers a complete solution to the problems of the inheritance of awn and lateral floret seems certain; but *H. inerme* fluctuates very readily under environmental change and for this reason the evidence obtained so far from its crosses has not been used in this paper. A study of the seasonal fluctuation of *H. inerme* and of the two "intermediums" (*H. Haxtoni* and *H. transiens*) is, as crosses have repeatedly demonstrated, essential to finality in the elucidation of the awn and lateral floret problems.

I. The Rachilla.

In the barleys two forms of rachilla are recognised. The first [(*a*) in Fig. 1] has long, stiff, pointed hairs which are inclined to the axis of the rachilla at a small but variable angle. The appearance of such a rachilla is suggestive of a diminutive ear of barley, the rachilla hairs corresponding to the awns of the ear.

The second type of rachilla [(*b*) in Fig. 1] has a longer and more cylindrical axis, and the hairs upon this axis are all short, fine, slightly curled, and woolly in appearance. Commonly this type is known as the "smooth" rachilla, the first type being called the "bristly" rachilla.

(*a*) (*b*)

Fig. 1.

Atterberg (1) uses rachilla character in his classification of the Barleys to separate the "distichum" group into sub-groups, and in connection with it he further employs a somewhat obscure and variable character, viz. the prickles or projections on the nerves or veins of the outer palea [the "zähnchen" of Körnicke (2)]. In the experimental results here recorded, these "projections" do not appear as a "character," for their variability renders sorting unreliable.

At first both the hairs (or bristles) and the axis of the rachilla were noted in all the F_1 and F_2 plants, but it became apparent that in certain cases referred to later the axis was liable to considerable and unaccount-

able fluctuation, and consequently inheritance results are recorded for hairs (or bristles) only.

In all cases observation was confined to the median florets, for these florets alone exhibit well-developed rachillae throughout all the different botanical classes of the barleys. The rachilla of the lateral floret reaches a stage of development which corresponds very closely with the general development of the floret itself. Thus in *H. hexastichum* the rachilla of the lateral is as well developed as that of the median. The "intermedium" barleys (*H. Haxtoni* and *H. transiens*) have full "bristly" rachillae on their medians, while the rachillae of the laterals are long and slender in the axis, the bristles being short and fine. In the "distichum" barleys the laterals are small and infertile, and, whether the rachillae of the medians be bristly or smooth, those of the laterals have hairs which are so diminutive that they are scarcely visible even under the microscope. Finally, in the "decipiens" barleys the lateral floret is reduced to two very small empty paleae and no trace of rachilla is to be seen.

Certain varieties of barley show very great fluctuation in the form of rachilla. In Praecox (*H. hexastichum*) in particular, a certain proportion of abnormal rachillae is always found. It not infrequently happens that only one rachilla on the whole ear is abnormal. Fig. 2 (*a*), (*b*), (*c*), (*d*), (*e*), (*f*) illustrates commonly occurring abnormal types in this variety. The normal type is the "bristly" (see (*a*) in Fig. 1).

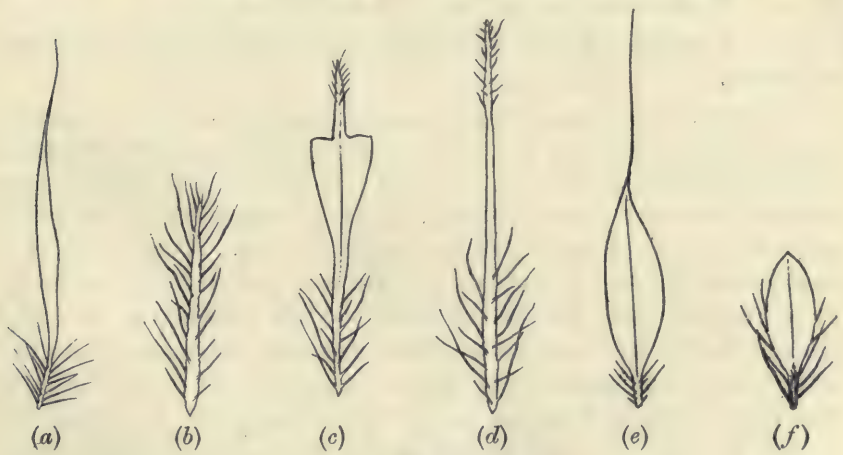

Fig. 2.

Some of the abnormalities present a "leaf-like" form and in the F_2 of a Praecox × Archer cross there occurred the type shown in Fig. 3,

in which the rachilla bore two "paleae." These paleae contained no traces of reproductive organs.

Fig. 3.

There are a few hairs on almost all parts of the barley plant. In the following regions hairs are abundant in varieties in general:

(i) Rachilla.

(ii) Glumes.

(iii) Awns (short, strong, spikes).

(iv) The thin edges of the rachilla segments.

(v) The lodicules (in the form of a fimbriation).

(vi) Roots (root hairs and therefore not strictly analogous to glume, etc. hairs).

A comparison between Archer and Chevallier barleys for example shows that the difference in the hairs of the rachillae exists, though it is not so marked, in (ii), (iii), (iv), and (v). In the root hairs (vi), no constant difference appears to exist. Thus it is suggested that one factor governs the type of hair in all the regions (i)—(v). Examination of a considerable number of barleys supported this view, but (iii) had finally to be removed from the list on account of the evidence of the "distichum" barley *H. d. persicum*. This sub-variety has smooth awns but both rachillae and glumes bear long bristly hairs. It is of interest to note that another well-known smooth-awned barley, *H. d. glabrum*, has smooth glumes, extremely short lodicule processes, and rachilla hairs which are far shorter and finer than those of Chevallier.

It is desirable to study this "parallel" development of the hairs of different regions and, in particular, to investigate the root hairs, for upon

them may depend some of the most important components of "yield." Investigations to this end are being undertaken. [The comparative morphology of the "hairs" on different parts of the plant may, perhaps, afford indications as to localities in which "parallel" development is to be expected.]

The undermentioned crosses supply data concerning the inheritance of type of rachilla.

Cross No. 53.

Russian Goldthorpe, No. 9 × Plumage
2-row, Bristly × 2-row, Smooth

F_1 = four plants; all bristly

F_2 = 188 plants. Bristly : Smooth = 144 : 44
= 76·6 % : 23·4 %

In this case the deviation from 3:1 expectation is less than half the standard error.

Cross No. 32.

Russian Chevallier, No. 7 × Inerme
2-row, Smooth × 2-row, Bristly

F_1 = 2 plants; both bristly

[The seeds of these plants were sown on separately and the F_2 families were designated 32/1 and 32/2.]

F_2	32/1 = 299 plants.	Bristly : Smooth = 220 :	79
	32/2 = 194 plants.	Bristly : Smooth = 149 :	45
F_2	Total = 493	Bristly : Smooth = 369 :	124

i.e. Bristly : Smooth = 74·8 % : 25·2 %

In this case the deviation from the 3 : 1 expectation is far less than the standard error.

Cross No. 57.

Praecox × Plumage
6-row, Bristly × 2-row, Smooth

F_1 = 3 plants; all bristly

F_2 = 186 plants. Bristly : Smooth = 146 : 40

This suggests, but is by no means closely in accordance with a 3 : 1 ratio. Observation and theory compare thus:

	Bristly	: Smooth
Observed	78·5 %	: 21·5 %
Calculated (3 : 1) ...	76 %	: 25 %

The deviation is thus about four times the standard error and cannot, therefore, with assurance, be regarded as a fluctuation of sampling.

The abnormal rachilla forms found in the Praecox parent (see above) made their appearance in F_2. They were not confined to any particular type or types of F_2 (as 6-row, 2-row, intermediate, etc.), but were found in a few cases in all the F_2 types. Abnormal forms were often difficult to classify, and this may possibly be the reason for the very considerable divergence from the 3 : 1 ratio—a ratio for which the remaining crosses vouch.

Cross No. 45.

Russian Chevallier, No. 7 × English Archer
2-row, Smooth | 2-row, Bristly
F_1 = all bristly
F_2 = 546 plants. Bristly : Smooth = 410 : 136
= 75·1 % : 24·9 %

The agreement with 3 : 1 expectation is thus extremely close.

Cross No. 35.

Russian Goldthorpe, No. 10 × Inerme
2-row, Smooth | 2-row, Bristly
F_1 = 30 plants ; all bristly
F_2 = 510 plants. Bristly : Smooth = 387 : 123
= 75·8 % : 24·2 %.

Here again, the deviation from 3 : 1 expectation is well within the standard error.

From the above results it is concluded that :

(*α*) One factor governs type of hair in rachilla.

(*β*) "Bristly" is dominant to smooth, the F_1 being apparently identical with the "bristly" P_1.

(*γ*) Possibly this same factor also governs the type of hair in the glume, rachis segment, and lodicule.

II. The Lateral Floret.

The barleys, i.e. the sub-species of *Hordeum sativum* Pers., are placed in primary groups in accordance with the forms of their lateral florets. In hybrid generations are encountered lateral florets of forms other than those which characterise the recognised "varieties" or sub-species of the barleys. To facilitate the description of hybrid generations there is given [(A)—(G) below] an account of all of the

forms of lateral floret which have been found in those F_2's and their parents, with which this paper deals.

The various forms of the lateral floret:

(A) Always fertile (i.e. forming a grain), the grain being nearly as large as that formed by the median floret. The lateral is awned or awnless in strict conformity with the median of the race concerned[1]. This form of lateral characterises *H. hexastichum* (the 6-row and 4-row barleys of the agriculturist).

(B) Always fertile under normal conditions but the grain decidedly smaller than that of the median floret.

Two sub-species, viz. *H. Haxtoni* and *H. transiens*, possess this form of lateral, and they constitute the race *H. intermedium*. In them, the outer palea of the median bears a long awn while that of the lateral is completely awnless, being rounded at the tip. Under adverse conditions it not infrequently happens that some of the laterals—or even all—fail to set grain. They are, nevertheless, quite perfect in stamens and ovary, and the non-fertility is purely a physiological response to environment; it has absolutely no genetic significance.

(C) Fertile; grain small, smaller than in the case of (B); outer palea terminating in an awn which may be merely a point or up to 3 cm. long. This type is confined to hybrid generations and appears to be a heterozygote.

(D) Non-fertile but inflated; the outer palea pointed or shortly awned. Like (C) it occurs only in hybrid generations and (see below) is probably a heterozygote. Stamens and a reduced ovary are present.

(E) Non-fertile but large and inflated; outer palea always rounded and devoid of an awn. Stamens and a reduced ovary are present. This, again, occurs only in hybrid generations.

(F) Non-fertile, of full length, but not inflated. The outer palea is always rounded and awnless. Stamens and a very small shapeless ovary are present. This type characterises the "distichum" barleys to which the familiar malting varieties Archer, Chevallier, Goldthorpe, etc. belong. Changes of environment, however pronounced, appear not to be able to cause this type of lateral to set grain.

(G) Non-fertile and extremely reduced. There is a small outer palea and a vestigial inner one, but no traces of rachilla or reproductive organs are to be seen. This is the Abyssinian or "decipiens" type.

[1] The 6-row type devoid of awns in both median and lateral florets is exemplified by the variety "Nogenasi" which Ikeno (9) employed in a cross to determine the inheritance of awns.

This classification of the forms of the lateral floret, although adequate for the recognised natural races of barley, cannot be infallibly applied to hybrid generations, even when types (C), (D) and (E) are employed. The late tillers of a single plant may differ from the early ones, and upon any ear more than one type of lateral floret may occur, e.g. (C) and (D), (D) and (E), etc.

The exact morphology of (F) and (G) will have to be determined by sections of developing ears at successive early stages.

Hordeum inerme—an awnless distichum obtained first by Rimpau from the cross *H. Steudelii* × *H. trifurcatum*—offers many complexities when used in crosses, in connection with the form of the natural floret. Results so far obtained upon certain F_2's in which *H. inerme* has been used, have not given absolutely consistent results, and consequently they are not included in this paper. *H. inerme* appears to be the key to the problem of inheritance of several characters, e.g. lateral floret and awns.

Details concerning the classification by form of lateral floret are to be found in any systematic account of the cereals. The most practicable classification appears to be that of Beaven (3).

III. The Inheritance of the Lateral Floret.

Observations have been made upon a considerable number of crosses between 6-row and 2-row (distichum) barleys. In the notation of § II above these may be represented by (A) × (F). In some cases reciprocal crosses were made, but as both directions of the cross gave the same result—a fact established by Biffen (4) and (5) for many barley crosses—the ♂ and ♀ parents are not separately designated in the tables of results.

At the outset, an attempt was made to classify in the F_2's all the various forms of lateral which were neither (A) nor (F). Table I

TABLE I. *Six-row* × *Two-row.*

No. of Cross		F_1	F_2 (type of lateral) (A)	(B)	(D) Awned	(D) Pointed	(F)
43	6-row Praecox × 2-row Archer	Laterals bearing a short awn. On every ear a few laterals set grains	129	9	80	171	118
57	6-row Praecox × 2-row Plumage	As for F_2 . 43	48	39	30	30	39
58	6-row Praecox × 2-row Archplume	Laterals large and sharply pointed but none set grain	108	47	←—Total = 280—→		

contains the results of some of the attempts, the notation for the types of lateral floret being that of § II above. It is apparent that the relations of the frequencies of the (B), (D)-awned, and (D)-pointed classes are not constant, and the conclusion is that these classes are not constantly and genetically different. In the light of the facts of fluctuation mentioned in § II (B) above, the simplest view to adopt is that these three classes are genetically identical, and that their apparent differences represent the effects of environmental irregularities. Biffen (4) in the case of the cross *H. Schimperianum* (6-row) × *H. nutans* (2-row) has completely justified this view by proving that all F_2 plants which were neither (A) nor (F) gave, in spite of eye differences *inter se*, F_3 families containing (A), (F) and various forms of the (D) type, i.e. that all such F_2 plants were heterozygous in respect to one factor, for form of lateral floret.

In consequence of this conclusion, the remaining F_2's were classified by grouping all the (A) plants and the (F) plants, and combining the balance of the F_2 under the name "heterozygote." Table II gives, in

TABLE II.

No. of Cross	Parents	F_2 types of lateral floret: (A) i.e. 6-row	Heterozygote	(F) i.e. 2-row
43	6-row Praecox	129	260	118
	×	25·4 %	51·3 %	23·3 %
	2-row Archer	±1·9 %	±2·2 %	±1·9 %
57	6-row Praecox	48	99	39
	×	25·9 %	53·1 %	21·0 %
	2-row Plumage	±3·2 %	±3·7 %	±3·2 %
1	6-row Trifurcatum	46	83	43
	×	26·7 %	48·3 %	25·0 %
	2-row Archer	±3·3 %	±3·8 %	±3·3 %
2	6-row Victoria	50	109	53
	×	23·6 %	51·4 %	25·0 %
	2-row Archer	±3·0 %	±3·4 %	±3·0 %
4	6-row verbessert	55	94	61
	×	26·2 %	44·8 %	29·0 %
	2-row Archer	±3·0 %	±3·4 %	±3·0 %
5	6-row Frühling's	49	123	55
	×	21·6 %	54·2 %	24·2 %
	2-row Archer	±2·9 %	±3·3 %	±2·9 %
6	6-row Nachte kleine	21	53	23
	×	21·7 %	54·6 %	23·7 %
	2-row Archer	±4·4 %	±5·1 %	±4·4 %
	Totals	398	821	392
		24·8 %	50·9 %	24·3 %
		±1·1 %	±1·2 %	±1·1 %

this form, the data of several crosses. In this table, for every cross, the first row shows the actual numbers of plants in the (A), heterozygote, and (F) classes. The second row contains these numbers expressed as percentages, while the third row shows the standard errors of these percentages due to fluctuations of sampling. The standard errors have been calculated on the basis of an expectation of 1 : 2 : 1, i.e. 25 °/₀ : 50 °/₀ : 25 °/₀. The table contains twenty-four percentages, and in five cases only does the difference between observed and calculated percentages exceed the standard error, whereas some seven cases might be expected. The cases are as follows:

Cross 57	...	21·0 °/₀	instead of	25 ± 3·2
Cross 5	...	21·6 °/₀	,,	25 ± 2·9
Cross 5	...	54·2 °/₀	,,	50 ± 3·3
Cross 4	...	41·8 °/₀	,,	50 ± 3·4
Cross 4	...	29·0 °/₀	,,	25 ± 3·0

It is not easy to sort such an F_2 for type of lateral floret. In stunted plants the difference between (F) and "heterozygote" is much obscured. Consequently there seems justification for stating that the results of the crosses indicate the proportions (A) : heterozygote : (F) = 1 : 2 : 1 in F_2. The combined results of the crosses (last row of Table II) show no deviation as great as the relevant standard error.

To summarise these conclusions it may be said that:

(*a*) Six-row and two-row (distichum) barleys differ in regard to form of lateral floret by one factor only.

(*β*) The heterozygotes of F_2 resemble the F_1 plants. Like them they are subject to fluctuations induced by environment. These fluctuations result in apparent differences, but constantly different groups cannot be found.

Blaringhem (10) has introduced novel views in connection with crosses between 6-row and 2-row barleys. In the appendix (p. 106) attached to this paper will be found some observations upon his views.

v. Ubisch (6) has made extensive observations upon 6-row × 2-row crosses and has formulated a hypothesis on the assumption of a two-factor difference between the parents in regard to form of lateral floret. He found that the laterals of the F_1 plants, like those of the F_2 heterozygote plants, were much influenced by conditions. The classification adopted for the F_2 was

zwei-zeilig : zwei- bis sechs-zeilig : sechs-zeilig.

The middle class was thus defined: "Es hat sich als zweckmässig zur Analyse herausgestellt, allen Typen, die gelegentlich fertil sind, als zwei- bis sechszeilig zu bezeichnen."

In the 2-bis-6 class were found several types, such as "Spitz," "Spitz verlängert," etc., and consequently, although he recognised that the F_2 ratios were monohybrid, he thought it desirable to employ a di-hybrid or 2-factor explanation, because of the diversity of the heterozygote forms. The fit of observation to expectation is good. As factors were employed: *Z*, which is responsible for the 2-row state; *W*, an intensifier which is operative only in the presence of *Z*, and in the absence of which there is an "Übergehen" from 2-row to 2–6-row. In the light of the evidence furnished by the crosses described in this paper, two points of v. Ubisch's theory appear not to harmonise with the facts of observation. These are:

(*α*) Plants of the constitution *ZZWw* and *ZZww* are described as 2 bis 2–6 zeilig. This seems to imply that 2-zeilig is not genetically a definite group. The evidence of Table II demands that it should be of definite and invariable genetic constitution.

(*β*) The ratio 6-row: all others is stated to be 1 : 3. In Table II the ratio 2-row: all others also appears to be 1 : 3. v. Ubisch however finds that the proportion of 2-row: all others is 3 : 13. This divergence from the results recorded in Table II is, of course, simply a consequence of (*α*) above.

IV. The Problem of Inheritance in Lateral Florets.

As described in § II, the lateral florets found in the various races of barley, and in their crosses, form a series (A)—(G). There is reason to believe that (B), (C), (D) and (E) are genetically alike in regard to constitution for the development of reproductive organs. Probably there occurs a further series, corresponding to (B), (C), (D) and (E), and produced by crosses between decipiens and distichum types (see below).

The evidence which has accumulated up to date concerning the general question of lateral floret inheritance in barleys seems sufficient to justify a brief review. Much of it has already been published by Biffen (4) and (5). In these publications will be found the details of those cases which are simply recorded in outline below. The four sub-groups of barley—hexastichum, intermedium, distichum, decipiens—together with the interesting awnless *H. inerme*, permit of ten sets of crosses, and these are now described serially:

(*α*) *Hexastichum* × *intermedium*, i.e. (A) × (B).

In all cases the F_1 had six complete rows of fertile florets, but the

laterals were less strongly awned than were the medians [Hexastichum barleys have their medians and laterals fully awned; intermediums have fully awned medians and completely awnless, blunt-ended laterals].

Cross No. 14—*Haxtoni* × *pyramidatum*—was worked out fairly completely. In the F_2 the following proportions were found:

Typical 6-row : Remainder of $F_2 = 43 : 133$

(all awns full) . . . $= 1 : 3$

The "remainder" consisted of some plants exactly resembling the F_1, of some with part only of their lateral florets fertile and with their lateral outer paleae sharply pointed, and of some with occasional fertile laterals, the lateral outer paleae being always blunt (as in the Haxtoni parent). From the F_3 it was clear that the "typical 6-row" of the F_2 was homozygous, as was the F_2 group which resembled Haxtoni. The F_2 group, whose lateral paleae were pointed, produced in F_3 all types (the two parental and the pointed lateral). They were therefore heterozygous. The variability of the shape and fertility of the lateral—common in heterozygotes—made precise sorting very difficult, and the proportion of Haxtoni type in F_2 could not satisfactorily be proved to be 1/4 (actually it was 21·1 %). That the Haxtoni type of F_2 bore some infertile laterals on certain (or all ears) of some plants, is in conformity with the fact that precisely the same thing may and often does occur in pure parental Haxtoni.

The definiteness of the 1 : 4 proportions of pure 6-row type in F_2 (43 plants out of an F_2 of 176 plants) and the approach of the proportion of Haxtoni plants in F_2 to 25 %, point to the fact that the difference between the parental forms is, for lateral floret, unifactorial.

It may therefore be stated—using the notation of § II above—that the lateral floret forms (A) and (B) are allelomorphs.

(β) *Hexastichum* × *distichum*, i.e. (A) and (F).

The evidence of § III shows that (A) and (F) are allelomorphic characters.

(γ) *Hexastichum* × *decipiens*, i.e. (A) and (G).

The data given by Biffen (5) show that in the F_2 a 1 : 2 : 1 ratio was obtained.

The F_3 growings afforded full confirmation of the fact that the F_2 contained, in addition to both the parental forms, one and only one heterozygote.

Hence (A) and (G) are allelomorphic characters.

(δ) *Hexastichum* × *distichum* (*H. inerme*).

Consistent results have not been obtained. The extreme fluctuation to which *H. inerme* is liable in response to climatic change no doubt largely accounts for this lack of consistency. One fact of great interest has, however, been established. In the F_2, the combination 6-row-awnless does not occur. The inter-relation between six fertile rows and the absence of awns which this fact implies, has yet to be discovered.

(ϵ) *Intermedium* × *distichum*, i.e. (B) × (F).

Several such F_2's were raised in the summer of 1919, but the extreme drought of the early part of the flowering period produced great fluctuation. The ears of the single plant differed markedly among themselves in some cases. It seems that here, too, a 1 : 2 : 1 ratio is likely to be found, but the complete F_3's of 1920 will have to be awaited for a confirmation of the anticipation.

(ζ) *Intermedium* × *decipiens*, i.e. (B) × (G).

Crosses between *H. gymnospermum* (awned, black, decipiens) and *H. Haxtoni* (typical intermedium) indicate that the F_2 consists of the two parental types, and a heterozygote type. Sorting is too difficult to give reliable proportions. The allelomorphism of (B) and (G) is not therefore properly established.

(η) *Intermedium* × *distichum* (*H. inerme*).

Cf. (δ) above. Here too emerges a fact of some interest. The proportion of awnless types is extremely low. The awn is subject to very great fluctuation, e.g. from ear to ear of a plant, and F_3's will be necessary before any definite result can be reached.

(θ) *Distichum* × *decipiens*, i.e. (F) × (G).

The 1 : 2 : 1 ratio has been well established; hence (F) and (G) are allelomorphic characters.

(ι) *Distichum* × *distichum* (*H. inerme*).

Only by complete F_3's (to be grown in 1920) can this cross be properly studied.

(κ) *Decipiens* × *distichum* (*H. inerme*).

No observations are available.

The above categories of crossing results may be summarised thus:

The following have been shown to be pairs of allelomorphic characters:

(A) and (B), see (α).
(A) and (F), see (β).
(A) and (G), see (γ).
(F) and (G), see (θ).

The following may also prove to be pairs of allelomorphic characters:

(B) and (F), see (ϵ).
(B) and (G), see (ζ).

The various forms of the lateral floret in barley thus appear to afford another instance of the phenomenon which is now known as "multiple allelomorphism." The work of Morgan on eye-colour in *Drosophila*, the coat-colour of mice, colour in the maize cob, silk, etc., and the experiments of Nabours (8) on *Paratettix* afford further evidence of this phenomenon—a phenomenon which, other things apart, is of great interest because of its failure to harmonise with the "presence and absence" hypothesis (in its simple form and without appeal to complex linkages).

Few instances of genetically related degrees of development appear to be so evenly graded as that which has been described here, and a good opportunity presents itself for an attempt to correlate "Mendelian characters" with morphological structures. Such an attempt would have to be based upon a histological study of the various forms of lateral floret at successive developmental stages. The difficulties imposed upon the sorting of F_2 types by fluctuations due to environment are very great, and it is unlikely that they will be completely surmounted without a histological study of this kind.

Appendix. Some observations upon the views presented by Blaringhem (10) *concerning the results of certain crosses between 6-row and 2-row barleys.*

Blaringhem has insisted upon the occurrence and upon the importance of "L'Hérédité en mosaïque" (or "Naudinienne"). He has cited the following 6-row × 2-row barley cross as an example.

H. tetrastichum pallidum was crossed with *H. distichum nutans*. The F_1 consisted of "plantes vigoureuses offrant toutes des épis à deux rangs." No more detailed specification of F_1 characters was recorded. In F_2 there were 410 plants. The 6-row type numbered 134, and they were "presque aussi nombreuses que les plantes à deux rangs"; but what was accepted as the most striking thing about these 6-row plants

from F_2 was the fact that at the base of the ear they were 6-row, while at the upper end they were 2-row. In other words, they represented "mosaic inheritance."

A number of objections have to be lodged against these conclusions:

(1) "à deux rangs" is, in such a case, too vague a description to carry any strict classificatory meaning. It has repeatedly been observed that the F_1 plants of a 6-row × 2-row cross while invariably possessing large fertile median florets, have variable laterals. The laterals may all be simply large, pointed, and inflated (as compared with those of genuine 2-row barley); but very commonly a certain small proportion of them on any ear sets grains. The variability is considerable, but the distinction from pure 2-row is always apparent. The descriptions employed are so vague that there arises doubt as to whether the precise differences between heterozygous and homozygous (2-row) were considered.

(2) The proportions of types recorded for the F_2 are such as do not appear among the numerous other recorded results.

Thus, 2-row : total $F_2 = 134 : 401$ (about).

From the statements made in the text the expectation is

$$100 : 400.$$

If plants of homozygous type, which owing to conditions had failed to set any lateral grains, were classed as "à deux rangs," then an excess of 2-row was certain to result. Moreover if *all* plants having *some* fertile lateral florets were regarded as "6-row," this category was certain to include some of the heterozygotes (which are like F_1) and again an excess over the 1 : 4 expectation is to be anticipated.

(3) In the text the extreme susceptibility of the sexual organs of potentially fertile lateral florets has been explained. The consequent variability—some fertile and some non-fertile laterals on the same ear—is so great, and so commonly exemplified in F_1's and F_2's, that without a very critical examination no conclusion as to "mosaics" seems warranted. Clearly every ear which Blaringhem classed as 6-row in his F_2 was not a "mosaic"; but the amount of mosaicism cannot be gauged from the record "presque toutes offraient la mosaïque des caractères."

(4) The opinion that the so-called "mosaics" were simply heterozygotes is confirmed by the fact that grains collected from the "2-row part of the ears" and also those collected from the "6-row part of the ears," both gave all three ear-types when sown.

(5) Variation in the form of the mosaic is yet another indication of ordinary lateral-floret "sterility" (better, "non-fertility"). Whereas, in general, the mosaic was of the type "6-row base and 2-row tip" there were found some ears 6-row all down one side and 2-row all down the other. It is not stated whether or not each kind of "mosaic" bred true and whether or not both types could occur upon a single plant. These questions of circumstantial fact are very important.

On p. 246 (10) a somewhat revolutionary conclusion is recorded. Crosses between closely allied lines exhibit Mendelian inheritance: those between distant lines ("du mélange d'espèces linnéennes distinctes—nutans, erectum, nudum") are characterised by ratios differing from those required by Mendel's Laws. These deductions were made with reference to crosses in which the experimental character was "projections on the nerves of the palea." It is to be observed that many barley crosses have been made and "Mendelian" results recorded, despite the fact that the parents were more distantly related than those which Blaringhem cited. However, the results recorded in connection with Blaringhem's particular character are not such as to afford, of themselves, a refutation of the above conclusions. [The inter-specific crosses among the wheats furnish perhaps the best collection of evidence in refutation of the conclusions.]

BIBLIOGRAPHY.

1. Atterberg. *Transactions of Swedish Horticultural Society*, 1905.
2. Körnicke. *Arten u. Varietäten des Getreides.* Berlin, 1885.
3. Beaven. "Varieties of Barley." *J. Fed. Inst. Brewing*, Vol. viii. No. 5. 1902.
4. Biffen. "The Hybridisation of Barleys." *J. Ag. Sci.* Vol. ii. 1907—8.
5. ——. "The Inheritance of Sterility in Barleys." *J. Ag. Sci.* Vol. i. 1905—6.
6. v. Ubisch. "Beitrag zu einer Faktorenanalyse von Gerste." *Zeit. f. Ind. Ab. u. Vererb.* B. xvii. H. 1/2. Nov. 1916.
7. Morgan. "Dilution Effects and Bicolorism in Certain Eye Colours of Drosophila." *J. Exp. Zoo.* xv.
8. Nabours. "Studies of Inheritance and Evolution in Orthoptera." *J. Gen.* iii.
9. Ikeno. "Étude génétique sur les arêtes d'une race de l'Orge à six rangs." *Bot. Mag.* Tokyo, xxxi. No. 370, 1919.
10. Blaringhem. *Les Problèmes de l'Hérédité expérimentale.* Paris, Ernest Flammarion, 1919.

THE INHERITANCE OF GLUME-LENGTH AND GRAIN-LENGTH IN A WHEAT CROSS.

By F. L. ENGLEDOW.

(With 1 Text-figure.)

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I. The Experimental Material and the Nature of the Breeding.

The inheritance results in wheat published by Biffen (1) in 1905, made it clear that certain simply "measurable" or "quantitative" characters behaved, in inheritance, in much the same general fashion as the "qualitative" ones. Manifestly most of the economic plant-breeding questions, e.g. "yield" inheritance, must depend upon "measurable" characters, and the investigations to be described were undertaken with the primary object of ascertaining the precise mode of inheritance of some simply measurable character. Biffen's observations on length

of glume had revealed the segregation of "long" and "short" glumes (apparently of the parental forms) in the F_2 and, in addition, of heterozygous forms of intermediate length. It seemed desirable to determine whether or not the numerical limits which characterised the parents of the cross, also characterised the apparently "parental" types found in the F_2.

Glume-length was chosen as the experimental character partly because of the general definiteness of Biffen's results, and further, on account of its botanical suitability to the requirements of a simply measurable character. It was such that numerous and accurate measurements were obtainable, and moreover, as a general experience proved, it was less liable to wide and sudden fluctuations than were most of the other length characters of the wheat-plant.

Grain-length it was clear, bore some definite relation to glume-length [vide (1), p. 38], and as it appeared to have an economic significance, it was included as an experimental character in the investigation.

The parents (F_0) of the cross were "Polish" (*T. polonicum* L.) and "Kubanka" (a variety of *T. durum* Desf.). They had been grown for some years by Professor Biffen, and were known to satisfy the requirements of a definition of "pure line" with regard to origin, behaviour, and mode of maintenance.

The sequence of the breeding was:

1911 { Cross made, viz. ♀ Polish × ♂ Kubanka.
Parental stocks continued.

1912 { F_1 plants grown and harvested.
Parental stocks continued.

1913 { Every grain borne by the F_1 plants was sown and 530 F_2 plants thus raised. The main ear of every F_2 plant was harvested and labelled with a serial number (1—530). Of these plants only 487 set ripe grain.
Parental stocks continued.

1914 { Every grain of every labelled ripe F_2 ear (one per plant) was sown, and 5145 F_3 plants were thus raised. The main ear of every plant was harvested. The ears from those plants which sprang from the seeds of any one F_2 plant were bunched together, and the bunch was labelled with a "family number" which was the number allotted in 1913 to the corresponding F_2 parent plant.
Parental stocks continued.

The investigation began in 1913. Prior to that year the material was in the hands of Professor Biffen.

A certain amount of sterility was encountered, but as the details given below make clear, its incidence was neither selective nor sufficiently heavy to bias the numerical results recorded.

In the F_2 a few plants failed to mature by harvest time, and consequently they were not measured. These again were not selective, but included all three of the length types found in F_2.

The F_3 plants were kept unthreshed during 1914—19, and it is hoped to raise an F_4 in 1920.

II. The Observations and the Method of Measurement.

The class-intervals of measurement are, throughout:

For glumes, 1·0 mm.; for grains, 0·5 mm.

As a fact, the glumes were measured to 0·5 mm., but on this basis the frequency distribution, in the case of Polish, covered a range of 45 classes. Hence to facilitate statistical work and to smooth out the irregularities introduced into such a distribution by very small classes, the observations were regrouped into 1·0 mm. classes. As a result of this regrouping a non-integral (x·5) number of individuals is to be found as the class frequency in some parts of the tables of observations.

In all the generations one ear per plant was harvested and one glume per ear was measured. The grain situated nearest to that glume was measured also. Either glume of the median spikelet on either side of the rachis (i.e. any one of a specified set of four glumes) was selected for measurement.

The acceptance of one glume-length as a characteristic of the ear concerned, was justified by a separate investigation. It was first established that the glumes of the median group of four spikelets on an ear, were all of practically the same length. Thus the average of this set of eight glumes (from four spikelets) could be regarded as a characteristic of the ear. Next, for every F_2 plant (total 492 plants) these eight glumes (and with them the corresponding eight grains) were measured. The frequency distribution of the deviation of every single measurement from the mean of the set of eight to which it belonged, was then compiled, regard being paid to the sign of the deviation. The mean quartile deviation of the distribution was 0·401 mm. by direct calculation and 0·435 mm. when calculated from the standard deviation of the distribution on the assumption of normality. That is to say, fifty per

cent. of the single measurements deviate from the means of the sets of eight to which they belong, by no more than ± 0·4 mm. The accuracy of measurement is only ± 0·5 mm., and consequently one glume measurement per ear was considered to be justifiable.

III. The Inheritance of Glume-Length.

The symbols "*P*," "*I*," and "*K*" are used to facilitate reference.

Thus "*P*" implies "Polish" or "of Polish type."

$F_0 . P$ designates a population of pure "parental" Polish type, the type which was employed as one parent in the cross.

$F_0 . M_P$ (glume) denotes the mean glume-length of a population of parental Polish plants.

$F_0 . \sigma_P$ (glume) denotes the standard deviation of the glume-length frequency distribution for such a population.

"*K*" is similarly used in the case of "Kubanka."

"*I*" implies the heterozygote form which, as later appears, is roughly-speaking intermediate between "*P*" and "*K*".

The glume-length distributions in successive generations were as follows:

F_0.

Both parent varieties were grown and measured in 1913 and 1914. For comparative purposes the results of 1914 are by far the more useful, for they are based on much greater numbers of observations, and the F_3 was also grown in that year, the conditions being precisely similar. Summarised, the results were:

Glume-lengths of Parent Varieties ($F_0 . P$ and $F_0 . K$) of the Cross.

Year	Variety	Mean Glume-Length	σ	No. of Observations	No. of Table containing frequency distribution of glume-length
1913	Polish	$F_0 . M_P = 28{\cdot}6 \pm 0{\cdot}34$	—	80	—
1914	,,	$F_0 . M_P = 30{\cdot}84 \pm 0{\cdot}10$	3·784	600	I
1913	Kubanka	$F_0 . M_K = 11{\cdot}23 \pm 0{\cdot}24$	—	25	—
1914	,,	$F_0 . M_K = 11{\cdot}70 \pm 0{\cdot}04$	1·260	400	II

That the variability of Polish is great and that of Kubanka small becomes clear from an inspection of Tables I and II.

F_1.

During 1914–19, the F_1 measurements were lost. From memory it can be stated simply that the F_1 mean lay somewhere between the parental means—probably it was close to the arithmetic mean of the

parental values. Backhouse (2) records extensive crosses between Polish-Kubanka, and the following values are from his results:

$$F_0 . M_P = 29{\cdot}0 \text{ mm.}; \; F_0 . M_K = 9{\cdot}0 \text{ mm.}; \; F_1 . M = 18\text{–}19{\cdot}0 \text{ mm.}$$

Similarly from the records of Biffen [(1), p. 36] for reciprocal Polish × Rivet crosses, the numerical intermediateness of the F_1 is quite definite.

In the cross to be described the F_1 value was thus probably

$$= (30{\cdot}84 + 11{\cdot}70)/2 = 21{\cdot}27 \text{ mm.},$$

but no further use is made of this speculative value.

F_2.

Although 530 F_2 plants were raised, only 487 of them furnished grain for the raising of an F_3. The causes and distributions of the failures are explained in the tables which follow. The frequency distributions of F_2 glume-lengths were:

Table III (p. 133). The complete F_2 of 530 plants.

Table IV. The 487 F_2 plants which matured and whose grain was sown to raise the F_3.

Table V. The 17 F_2 plants which, although they contributed no grain for the F_3 sowings, did set one or more imperfect grains. They were unripe at harvest.

Table VI. The 7 F_2 plants which matured but were sterile.

Table VII. The 19 F_2 plants which were unripe at harvest, and which gave no evidence of being able to set grain.

It is with the plants of Table IV that further observation is concerned, for they alone gave F_3 progeny, from the examination of which they themselves could be sorted into genetic classes. Table IV (like Table III) exhibits a definite tri-modality.

Two conclusions may be drawn from the F_2 glume-length frequency distributions (Table III or Table IV) viz.:

(1) Three glume-length types appear in F_2.

(2) None of these types corresponds exactly to the Polish parent. It appears that this parental type is represented in the F_2 by a group of plants whose mean and fluctuation ranges for glume-length have been "shifted" down towards the lower end of the scale.

In the first place an attempt was made to "dissect" the tri-modal F_2 distribution into its three constituent types by purely mathematical methods. It was assumed that the distribution of the heterozygote type of F_2 did not extend below the lowest "peak" of the total F_2

distribution (i.e. 11·5 mm. in Table IV). If this were so, then that part of Table IV lying between 11·5 mm. and 9·5 mm. (both values inclusive) was the lower half of the distribution of all the plants of Kubanka type in the F_2. The upper half of the distribution was obtained by writing down the frequencies symmetrical about 11·5 mm. to those of the lower half. From the distribution so completed the standard deviation (σ) and number of observations (N) were found, and the mean M was, of course, 11·5 mm. The "normal" curve equivalent to this distribution was calculated [see Yule (5) for formula and tables for use in calculation]. By the same processes the normal curve for the distribution of the Polish type of F_2 was obtained. Subtraction of these two normal distributions from the total F_2 gave the distribution of the heterozygote or intermediate type. Thus calculated, the frequencies of the F_2 types were:

K	*I*	*P*
23·29 %	57·76 %	18·94 %:

while the modes were:

11·5 mm.	16·5 mm.	24·5 mm.

This theoretical "dissection" was carried out for the sole purpose of testing the reliability of mathematical analysis of the F_2 as against the far more lengthy and laborious genetic analysis by means of a complete F_3.

Clearly, the analysis of F_2 into constituent genetic types necessitated the raising of a complete F_3.

F_3.

The F_3 plants were kept in "families," an F_3 family consisting of all the plants raised from the grain borne by any one F_2 "mother" plant. From the nature of the F_3 family the genetic type of the corresponding F_2 plant was judged. Thus an F_3 family in which all the plants were P, must have originated from a homozygously P plant of the F_2; similarly in the case of K. An F_3 family containing plants of two or more types, e.g. P, I, and K, or P and I, or I and K, or P and K, must have come from an F_2 plant that was heterozygous, i.e. an I plant.

The sorting into types of the F_3 families necessitated a definition of the three types P, I, and K (the tri-modality of the F_2 distribution pointed to the existence of only three types). Measurement as opposed to eye-judgment was intended to be the essence of this investigation, and an attempt was made to set up measurable (quantitative) type

standards. Table II suggests that an F_3 family should be regarded as K if, and only if, no plant in it was of greater glume-length than 14·5 mm. Since the accuracy of measurement was only ± 0·5 mm., and the class interval was 1·0 mm., the value of 15·5 mm. was finally adopted as an upper limit defining the K type of F_3 family.

From the records of observations, the serial numbers were noted of all F_3 families in which occurred no plant of glume-length greater than 15·5 mm. These families were inspected, and every plant of every family proved to be to the judgment of the eye—at this stage fairly practised—of K type in general appearance. Thus far, therefore, the metrical standard was applicable; moreover it harmonised with eye-judgment.

For the Polish (P) standard it was natural to turn to Table I ($F_0 . P$ distribution) and, for the time being, ignoring the outlying observation at 12·5 mm., to adopt 19·5 mm. (20·5 − 1·0) as the lower limit for the P type. F_3 families would thus be accepted as P if, and only if, they contained no plant of glume-length less than 19·5 mm. Certain F_3 families were found which, although they failed to pass this numerical test, were obviously composed entirely of plants possessing the unmistakable Polish eye-appearance. The "shift" downwards in glume-length exhibited in F_2 had persisted in F_3, and thus had rendered inapplicable the metrical "P" standard derived from the distribution of $F_0 . P$. A fresh metrical standard was essayed in the form of the total range of glume-length variation in F_3 families. This too failed to harmonise with facts of eye-judgment—facts so defined that they could not be ignored.

After full trial, the metrical standard was abandoned in the case of Polish, and in its stead eye-judgment was adopted. Polish and Kubanka are so well characterised in glume-form that the practised eye has little difficulty in separating the one type from the other and from heterozygotes (intermediates). The intermediates proved to be in every one of the numerous glume (and grain) characters, excellent "blends" of the parent forms.

The standards thus finally adopted were applied, every F_3 plant being twice judged. The second judgment accorded with the first and the result was:

$$F_3 = \left\{ \begin{array}{lll} 114\ K & \text{families} = 1237 & \text{plants} \\ 267\ I & \text{,,} = 2854 & \text{,,} \\ 101\ P & \text{,,} = 1054 & \text{,,} \end{array} \right\} = 5145 \text{ plants.}$$

Regarding all F_2 plants as simple heterozygotes, if in their F_3 families

there occurred more than one type, and denoting the heterozygote by "*I*," the F_2 ratio is:

$$F_2 = K : I : P = 114 : 267 \quad : 101 \text{ [= 482 plants]}$$
$$= 23{\cdot}65\ \% : 55{\cdot}39\ \% : 20{\cdot}95\ \%.$$

[N.B. 487 F_2 plants set good seed, but only 482 F_3 families were obtained at harvest.]

From the F_2 distribution it is concluded that there are three distinct F_2 types. The classification of the F_2 plants by means of the F_3 should, on the assumption of a one-factor difference between *P* and *K*, reveal a 1 : 2 : 1 ratio among these types. Actually the ratio (given above) deviates considerably from this expectation, and as Tables V, VI, and VII show, the deviation is not explicable on the basis of selective mortality or sterility. Confirmatory evidence is to be found in Biffen [(1), p. 36] with Polish × Rivet, Backhouse (2) with Polish × Kubanka, and Caporn (3) with Polish × Eloboni. In all three of these cases the evidence for a one-factor difference for glume-length was very substantial, and close approaches to a 1 : 2 : 1 ratio were obtained in the F_2. No other explanation appears to accord as closely with the results of observation above described as does that of one-factor difference; and consequently as a working hypothesis it is assumed that either *P* or *K* possesses a factor for glume-length which the other lacks, and that the heterozygote is, broadly speaking, intermediate between the parental forms.

IV. The Measurable Characteristics of the *P*, *I*, and *K* Glume Types in F_0, F_2, and F_3.

In F_2 and F_3 every plant was classified as *P*, *I*, or *K*. The next step was to evaluate the measurable characteristics—frequency distribution, mean, and standard deviation—of all three types in F_0, F_2, and F_3 successively. The *P* (and similarly *K*) plants of F_3 are of two kinds, the one descendants of $F_2.P$ (designated $F_3.P$ ex $F_2.P$) and the other of $F_2.I$ (designated $F_3.P$ ex $F_2.I$). For the time being the characteristics of $F_3.P$ ex $F_2.I$ and $F_3.K$ ex $F_2.I$ will not be considered. The measurable characteristics of the three types in the three generations were as follows:

M = mean glume-length; σ = standard deviation of glume-length frequency distribution and N = the number of observations in the distribution.

K.

Measurable Characteristics of the Kubanka Glume Type in F_0, F_2, and F_3.

Year	Generation	M	σ	N	Table of Frequency Distribution
1914	$F_0 . K$	$11{\cdot}70 \pm 0{\cdot}043$	1·26	400	II
1913	$F_2 . K$	$11{\cdot}42 \pm 0{\cdot}075$	1·18	114	VIII
1914	$F_3 . K$ ex $F_2 . K$	$11{\cdot}98 \pm 0{\cdot}025$	1·30	1237	IX

The $F_2 . K$ population was small (114 plants) and the 1913–14 seasonal difference was doubtless responsible to a considerable extent for differences in growth. From the above values it is concluded that the K type maintained its measurable characters practically constant in F_0, F_2, and F_3.

P.

Measurable Characteristics of the Polish Glume Type in F_0, F_2, and F_3.

Year	Generation	M	σ	N	Table of Frequency Distribution
1914	$F_0 . P$	$30{\cdot}84 \pm 0{\cdot}104$	3·78	600	I
1913	$F_2 . P$	$23{\cdot}18 \pm 0{\cdot}186$	2·75	101	X
1914	$F_3 . P$ ex $F_2 . P$	$24{\cdot}66 \pm 0{\cdot}078$	3·78	1054	XI

As in the case of Kubanka, the mean of (F_3 1914) is slightly greater than that of F_2 (1913) and the difference is small enough to be attributable to season.

The differences

$$F_0 . M_P - F_2 . M_P = 7{\cdot}66 \text{ mm.} = 24{\cdot}83\,\%_0 \text{ of } F_0 . M_P$$

and

$$F_0 . M_P - F_3 . M_P = 6{\cdot}18 \text{ mm.} = 20{\cdot}04\,\%_0 \text{ of } F_0 . M_P$$

are so great in comparison with the relevant probable errors that there is full justification for stating that in the $P \times K$ cross, the parental P does not re-appear in F_2. In its place are found P plants which closely resemble $F_0 . P$ in general appearance but whose mean glume-length is more than 20 % lower than that of $F_0 . P$. This "reduced" form of P in F_2 produces, when selfed, P plants of the same "reduced" glume-length in F_3. The possibility that "minor multiplying factors" are responsible in some way for this phenomenon of "reduction" or "shift" demands, perhaps, some form of reservation. It may be that the true $F_0 . P$ type is reproduced in F_2 but in extremely small proportion. Apart from the value of a safeguarding reservation there is nothing to recommend any theory of multiplying factors in this case. On the grounds of simplicity and agreement with observation, the most acceptable theory is that of the segregation of one factor with the attendant phenomenon of "shift."

Interesting evidence of the occurrence of "shift" accompanying an otherwise apparently single segregation of one factor is to be found in the results of a number of investigations. Caporn [(3), p. 259] has remarked upon it and it is clearly shown in some of Backhouse's results [e.g. (2), p. 130; Fig. 1*a*]. In both of these cases Polish was one parent of the cross. The importance of a recognition of "gametic impurity" or "contamination" has been emphasised by Ruggles-Gates (9) and in a variety of crosses the occurrence of the phenomenon is exemplified. Among these are Punnett and Bailey (10) and (11), Castle and Phillips (12), Davenport (13), Martin Leake [(14)—leaf factor in Cotton], East (15), Balls [(17), p. 69 on seed-weight], and possibly some of the intangible facts discovered in Tomato investigations by Groth (7) might be explained as results of "shift." It is interesting to observe that the results recorded by Belling (16) constitute one of the closest studies of length inheritance and that they show complete absence of "shift."

I.

Measurable Characters of the Heterozygous Glume Type in F_0, F_2, and F_3.

Year	Generation	M	σ	N	Table of Frequency Distribution
1913	$F_2 . I$	$16{\cdot}407 \pm 0{\cdot}078$	1·89	267	XII
1914	$F_3 . I$ ex $F_2 . I$	$17{\cdot}480 \pm 0{\cdot}058$	2·09	580	XIII

From Tables XII and XIII it appears that the frequency distribution of glume-lengths for the plants of F_2 and F_3 which are neither P nor K (and are therefore classed as I) is unimodal, and this is in accordance with the one-factor explanation which has been adopted. With regard to the relation of the mean glume-length of the heterozygote to the means of the parents, the following values are of interest.

$$(F_0 . M_P + F_0 . M_K)/2 = 21{\cdot}27\,\text{mm.} \quad (F_0 . M_P \times F_0 . M_K)^{\frac{1}{2}} = 19{\cdot}0\,\text{mm.}$$

$$(F_2 . M_P + F_2 . M_K)/2 = 17{\cdot}30\,\text{mm.} \quad (F_2 . M_P \times F_2 . M_K)^{\frac{1}{2}} = 16{\cdot}27\,\text{mm.}$$

$$F_2 . M_I = 16{\cdot}41\,\text{mm.}$$

It will be observed that a modified form of the "golden mean" theory of Groth (7) and (8) appears to fit the facts of this case.

The complete F_3 progeny of the $F_2 . I$ plants should, on the hypothesis adopted, present a glume-length distribution similar to the distribution of the complete F_2. That this is the case is shown by Table XIV. There appear to be three constituents with modes approximately at:

11·5 mm. 17·0 mm. 24·5 mm.

On expectation, these constituents are *K*, *I*, and *P*, and in order to determine their measurable characteristics, all plants of all $F_3 . I$ families (between family No. 51 and family No. 265 both inclusive) were re-measured and re-classified as *K*, *I*, or *P*. From a sample of 1190 plants thus obtained the following values were calculated :

Measurable Characters of the K, I, and P Glume Types bred from $F_2 . I$ plants.

Year	Generation	*M*	σ	*N*	Table of Frequency Distribution
1914	$F_3 . K$ ex $F_2 . I$	$11{\cdot}81 \pm 0{\cdot}053$	1·35	292	XV
,,	$F_3 . I$ ex $F_2 . I$	$17{\cdot}48 \pm 0{\cdot}058$	2·09	580	XIII
,,	$F_3 . P$ ex $F_2 . I$	$24{\cdot}68 \pm 0{\cdot}141$	3·73	318	XVI

Comparison of these values with those given under *K* (p. 117) and *P* (p. 117) show that for the mean glume-lengths:

$$[(F_3 . K \text{ ex } F_2 . K) - (F_3 . K \text{ ex } F_2 . I)] = 11{\cdot}98 - 11{\cdot}81 = +0{\cdot}17,$$

$$[(F_3 . P \text{ ex } F_2 . P) - (F_3 . P \text{ ex } F_2 . I)] = 24{\cdot}66 - 24{\cdot}68 = -0{\cdot}02.$$

That is to say, although "shift" was first exhibited by the *P* descendants of heterozygous plants (viz. by $F_2 . P$ ex F_1) it is not again exhibited by the *P* descendants of F_2 heterozygotes (viz. $F_3 . P$ ex $F_2 . I$). It appears that the reduced or "shifted" value of Polish glume-length cannot be further shifted by either

(α) Selfing—for $F_3 . P$ ex $F_2 . P$ (mean) $\triangleq F_2 . P$.

(β) Hybridisation, as in the zygosis performed by $F_2 . I$ plants—for $F_3 . P$ ex $F_2 . I$ (mean) $\triangleq F_3 . P$ ex $F_2 . P$.

V. The Inheritance of Grain-Length.

It was originally intended to deal with grain-length entirely by measurement but the principle of metrical type-standards proved inapplicable just as it did in the case of glume-length. In fact the breakdown was more complete, for the parental (*K* and *P*) mean grain-lengths lie, comparatively, very close, and the overlap of their distributions is considerable. From the early stages of the work upon F_2 it was quite certain that "*P*" grains were never associated with "*K*" glumes or *vice versâ* and after extensive trials of sorting grains into types, it was concluded that in F_2 three types only were found (*K*, *I*, and *P*), and that on any plant *K* glumes were invariably accompanied by *K* grains and similarly for *I* and for *P*. Consequently in F_2 and F_3 the classification by glumes was accepted as a classification by grains. Biffen [(1), p. 38] remarked upon the relationship between grain and glume.

In what follows the phrase "F_2. K grains" refers to the grains borne by those F_2 plants which have K glumes and so on, the plant categories in F_2 and F_3 being thus identical with those of the glume-classification already described. On account of shed-grain etc., the number of plants in a grain-category is sometimes slightly less than the number in the otherwise identical glume-category.

In the successive generations the grain-length distributions were:

F_0.

Grain-Lengths of Parent Varieties (F_0. P and F_0. K) of the Cross.

Year	Variety	M	σ	N	Table of Frequency Distribution
1913	Polish	$F_0 . M_P = 10{\cdot}8 \pm 0{\cdot}102$	—	80	—
1914	,,	$F_0 . M_P = 10{\cdot}2 \pm 0{\cdot}019$	0·66	526	XVIII
1913	Kubanka	$F_0 . M_K = 8{\cdot}2 \pm 0{\cdot}077$	—	25	—
1914	,,	$F_0 . M_K = 7{\cdot}7 \pm 0{\cdot}017$	0·49	395	XIX

The grain-length distributions are more smooth than those of glume-length and K again shows less dispersion than P.

F_1.

Like the F_1 glume measurements, those of the grains were lost. From memory and from previously published investigations it is concluded that the heterozygote length is roughly intermediate between the parental ones. In the F_2 the intermediate or heterozygote grain can, with practice, always be distinguished from P and K. In some five or six well marked characters, it is a very even blend or intermediate between the characters of the parent grain-forms.

F_2.

In maize, the occurrence of "double fertilization" has been established. Assuming it in wheat, the expectation is that the endosperm of the grain (like the embryo) will be one generation ahead of the plant which bears it. Later on the question is more fully considered but for the present the grain borne by an F_x plant will be referred to as F_x grain. This convention is in no sense prejudicial and it simplifies the designation of the grains of different generations.

Table XX contains the grain-length frequency distribution for the complete F_2. Comparison with Table XVIII (F_0. P) and Table XIX (F_0. K) indicates that:

(α) The F_2 distribution contains no constituent exactly like F_0. P for the frequency at 10·2 mm. is almost zero ($F_0 . M_P = 10{\cdot}2$ mm.).

(β) Similarly it contains no component exactly like

$$F_0 . K (F_0 . M_K = 7{\cdot}7 \text{ mm.}).$$

(γ) The F_2 distribution is unimodal but (α) and (β) suggest that 'shift" has occurred. Possibly therefore the distribution consists of three constituents which lie very close on account of shift and which, by marked overlap, produce unimodality of the whole.

If the grain be one generation ahead of the plant which bears it, the ratio in F_2 should be 3 : 2 : 3; if not a 1 : 2 : 1 is expected. The ratio is investigated in § VI below.

It is to be observed that the F_2 distribution is based entirely on measurements and consequently the phenomena of "shift," "unimodality," etc. are unaffected by the fact that in sorting it will be assumed that K (or I or P) grains always accompany K (or I or P) glumes only.

F_3.

The measurements and numbers of plants correspond with those for glume-length in § III above. Owing to the sterility of a few F_3 plants the distribution totals differ in some cases from those for the corresponding glumes.

VI. The Measurable Characteristics of the K, I, and P Grain Types in F_0, F_2, and F_3.

It is necessary to recall that "K grain" (or I or P) implies the grain borne by plants whose glumes have been classed by eye as "K" (or I or P). That is to say, glume-length and grain-length are assumed to be "genetic inseparable," to be governed by the same factor. Eye-impressions amply support the assumption, and its validity may now be tested. Glume-length showed segregation in F_2, as was made clear by examination of the F_3 progenies of the F_2 plants. The vindication of the assumption necessitates a demonstration of the segregation of grain-length in F_2 similar to that given for glume-length. This is afforded by the measurable characteristics of the K, I, and P grain types in F_0, F_2, and F_3, which were as follows:

K.

Measurable Characteristics of the Kubanka Grain Type in F_0, F_2, and F_3.

Year	Generation	M	σ	N	Table of Frequency Distribution
1914	$F_0 . K$	$7{\cdot}70 \pm 0{\cdot}017$	0·49	395	XIX
1913	$F_2 . K$	$8{\cdot}33 \pm 0{\cdot}042$	0·64	112	XXI
1914	$F_3 . K$ ex $F_2 . K$	$8{\cdot}19 \pm 0{\cdot}012$	0·63	1214	XXII

Tables XIX, XXI, and XXII show that the $F_0 . K$ type is not reproduced in F_2 as does the equation $F_2 . M_K - F_0 . M_K = 0{\cdot}63$ mm. An upward "shift" in mean glume-length appears to have occurred, and the shifted value to have bred true, for despite the 1913–14 seasonal difference, $F_2 . M_K$ and $F_3 . M_K$ differ by 0·14 mm. only. It will be recalled that in the case of K glume-length the differences concerned were so small that they could not safely be accepted as evidence of shift.

P.

Measurable Characteristics of the Polish Grain Type in F_0, F_2, and F_3.

Year	Generation	M	σ	N	Table of Frequency Distribution
1914	$F_0 . P$	$10{\cdot}20 \pm 0{\cdot}019$	0·66	526	XVIII
1913	$F_2 . P$	$8{\cdot}84 \pm 0{\cdot}038$	0·56	101	XXIII
1914	$F_3 . P$ ex $F_2 . P$	$8{\cdot}75 \pm 0{\cdot}026$	0·83	1028	XXIV

The tables of frequency distribution and the values of M show that $F_2 . P$ very closely resembles its F_3 progeny, but that both of them are definitely "shifted" down from $F_0 . P$. The amount of the shift is:

$$F_0 . M_P - F_2 . M_P = 1{\cdot}36 \text{ mm.} = 13{\cdot}34\ \%\ \text{of}\ F_0 . M_P,$$

$$F_0 . M_P - F_3 . M_P = 1{\cdot}45 \text{ mm.} = 14{\cdot}21\ \%\ \text{of}\ F_0 . M_P.$$

That there is a segregation of grain types in F_2 is shown by the regularity and resemblance of the distributions etc. of $F_2 . P$ and $F_3 . P$ (cf. also $F_2 . K$ and $F_3 . K$).

I.

Measurable Characteristics of the Heterozygous Grain Type in F_0, F_2, and F_3.

Year	Generation	M	σ	N	Table of Frequency Distribution
1913	$F_2 . I$	$8{\cdot}67 \pm 0{\cdot}026$	0·62	266	XXV

Thus the classification of the grains of the F_2 and F_3 plants by means of the glumes with which they are associated, has led to the conclusion that three grain types occur in F_2. These types naturally have the same frequency relations as the glume types, and as pointed out in § IV above the evidence indicates that the relations are actually an experimental expression of a 1 : 2 : 1 ratio. A unimodal distribution (Table XX, i.e. grain-lengths of the complete F_2) has thus been shown to consist of three overlapping separate constituents K, I, and P, the overlap having been much accentuated by the "inwards" shift of P and K from the F_0 values.

In the glume-length results of § IV (under "I") it was shown that the progeny of the $F_2 . I$ plants had a length distribution similar to

that of the complete F_2 (i.e. the progeny of F_1, which is also "I"). Table XXVI is for the grains of the complete descendants of the $F_2 . I$ plants, and its general resemblance to Table XIX furnishes an analogous proof in the case of grain-length.

In connection with glume-length a special series of measurements was made to ascertain if the means of $F_3 . P$ ex $F_2 . P$ and of $F_3 . P$ ex $F_2 . I$ were the same (and similarly for K). Some of the data of a separate investigation may be used as a similar test in the case of grain-length. In F_3 families Nos. 1–50 (both inclusive) and 450–494 (both inclusive) *every* grain in the ear was measured for plants ex $F_2 . P$ and for all P plants ex $F_2 . I$. The results were:

Year	Generation	M	σ	N	Table of Frequency Distribution
1914	$F_3 . P$ ex $F_2 . P$	$8{\cdot}532 \pm 0{\cdot}010$	1·03	4396	XXVII
,,	$F_3 . P$ ex $F_2 . I$	$8{\cdot}389 \pm 0{\cdot}013$	0·79	1644	XXVIII

Both of the values of M are less than the value given previously for $F_3 . P$, for the last named was based upon the measurement of one "outside" grain per ear. The inner grains of a spikelet being smaller than the outside ones lower the value of M.

The difference shown, viz. $8{\cdot}532 - 8{\cdot}389 = 0{\cdot}143$ mm., is small in comparison with the relevant probable errors, but, as compared with the values of M to which it relates, it appears to be not sufficiently significant to imply any difference in type between $F_3 . P$ ex $F_2 . P$ and $F_3 . P$ ex $F_2 . I$.

The conclusions so far reached may be thus briefly stated:

(α) The one factor which governs the P-K glume difference also governs the P-K grain difference.

(β) "Shift" occurs, so that the P and K types as seen in F_2 (glume and grain) are numerically "shifted" forms of the true F_0 type.

The shifted values persist in F_3.

(γ) In spite of the demands of the "double fertilization" theory, the grain appears in so far as its length is concerned to belong to the same generation as the plant on which it is borne, i.e. grain-length is a maternal character and segregates on the 1 : 2 : 1 basis in F_2.

VII. Concerning the Apparent Genetic Inseparability of Certain Characters in the Cross.

The glumes of parental P plants are of a definite, recognisable, general form; their grains are equally characteristic. F_2 or F_3 plants whose glumes are P, invariably have grains which also, to the eye,

are *P*. Moreover, no plants are found, in any generation, whose glumes are other than *P* and yet whose grains are *P*. That is to say the *P*-form of glume and the *P*-form of grain appear to be inseparables, remaining associated throughout the vicissitudes attendant upon the production of F_2 and F_3 plants. Such characters may be called "genetic inseparables" [cf. "Correlation" as used by Collins (4)]. For the *K* type the same association prevails. Heterozygous plants (*I*) present an appearance best described as a skilful blend of the *P* and *K* forms. With *I* glumes, *I* grains are always found.

Glume-form or appearance, like grain-form, is the resultant of a number of characteristics and since the resultants are inseparable, so must the constituents be. Close inspection of a great number of plants (F_0–F_3) leads to the conclusion that there is a considerable number of such inseparable constituent characters, and a list of them is given below. The simplest view of this matter is that one factor controls all the inseparables. The list is:

(1) Length of glume.

(2) Ribs on main lamina of glume.

(3) Shape of tip of glume.

(4) Curvature of keel of glume.

(5) Consistency of material of glume (*P* is "papery," *K* is more rigid).

(6) Length of grain.

(7) Shape and size of cross-section of grain.

(8) The angle at which the embryo of the grain is set into the endosperm.

(9) Number and length of the hairs at the apex of the grain.

(10) Distinctness of the outline of the apical pad of the grain ("Gipfel-polster"—Kcke).

For every one of these characters *I* proves to be an interparental blend.

To test the closeness of the association, the correlation between glume-length and grain-length was evaluated for $F_0 . P$ and $F_0 . K$. If r = coeff. of correlation:

$$(F_0 . K) . r = + 0{\cdot}348 \pm 0{\cdot}044 \ (N = 395),$$

$$(F_0 . P) . r = + 0{\cdot}392 \pm 0{\cdot}037 \ (N = 526).$$

The values of the coefficient are, at first sight, surprisingly low. It is to be borne in mind however that circumstances of environment which affect grain-length, do not of necessity exercise a proportionate or

indeed any influence upon glume-length. Some two months before harvest the ear of the wheat plant has fully emerged from the sheath and, whereas its glumes have attained practically full development, the grain is just commencing to form—fertilization having, it may be supposed, just taken place. Two plants of the same glume-length may thus, if their post fertilization environments be markedly different, develop grains of distinctly different length, whereas under identical conditions they would, presumably, have developed grains of the same length.

Whatever may be the explanation of the low values of r, it is to be expected that if classifying by glume-length automatically ensures grain-length classification, then the value of r will be the same for P (or K or I) in F_0, F_2, and F_3. As a test, r was evaluated for $F_2 . P$. The first of the eight pairs of measurements made in every F_2 plant (see § II above) was used, i.e. first glume-length and first grain-length. Calculation gave:

$$(F_2 . P) . r = + 0{\cdot}301 \pm 0{\cdot}090,$$

and it may be noted that:

$$(F_0 . P) . r - (F_2 . P) . r = + 0{\cdot}09 = \text{p. e. of } (F_2 . P) . r.$$

Thus r glume/grain appears to have reasonable constancy and therefore although the absolute value of r is not great, a constant relationship between glume-length and grain-length is inferred. Owing to the fact that glume development is not contemporaneous with that of grain, the relationship is liable to be concealed by environmental influences, particularly when it is expressed as a coefficient of correlation.

VIII. An Apparent Relationship between Glume-Length and the Development of Hairs on the Glume.

Both Polish and Kubanka are, from the point of view of ordinary descriptive botany, regarded as smooth-glumed wheats. As a fact each, and in particular $F_0 . P$, exhibits a slight development of hairs on the glumes. In F_2 there were found a few plants decidedly hairy and very noticeably different in this respect from the parents. All plants more hairy than $F_0 . P$ were noted and the distribution of their glume-lengths is given in Table XXIX. They numbered 56/494, but the data available do not permit of any particular construction being placed upon this ratio. It will be observed that the increased development of hairs appears to have taken place in association with glumes of lengths below the length range of $F_0 . P$. This fact and the impression gained from an examination of the glume hairs of great numbers of $F_0 . P$ plants, is in agreement with the finding of Backhouse (2) that "length of glume has

acted as an inhibitor of pubescence." Confirmatory evidence cannot be sought from the F_3 plants, for it is found that, comparatively quickly, glume hairs fall off or become so brittle that they are very readily rubbed off in handling, and the F_3 plants have been stored for five years.

IX. The Inheritance of Solidness of Straw in the Cross.

When the "shift" in glume-length became apparent, it was decided to investigate some character unconnected with glume- or grain-length, to see if it manifested in F_2 any feature corresponding to the shift of glume-length. The straw was selected. *P* is solid, *K* is hollow, and it is noteworthy that in all cases in which "shift" of glume-length is observable [vide cases cited in § IV above], one parent was solid in straw and the other hollow. Absence of shift characterises one case only, viz. the Polish × Rivet cross [Biffen (1)], and in this both the parents are solid in straw. Thus the nature of the straw might possibly be related to the shift in glume-length.

Biffen (1) for a Turgidum (solid) × Vulgare (hollow) cross (Rivet and Red King were the varieties actually used) found a sharp 3 : 1 = hollow : solid in F_2. In the cross here described it was difficult on many occasions to separate hollow and solid straws. "Intermediate-solids" were encountered and finally were included as solids. The F_2 was classified for straw by examination of its F_3 progenies, but in all three of the glume types (*P*, *I*, and *K*) the ratios were such as could not be ascribed to ordinary factor segregation, whether of one or more than one factor. Different ratios were found for every one of the glume-type constituents (*K*, *I*, and *P*) and a different one, again, for the complete F_2.

The result is therefore negative—shift of glume-length is not connected with any simple peculiarity of straw inheritance—but the fact that unaccountably irregular ratios were obtained points to the need of a continuation of the straw investigation in subsequent generations.

X. Evidence concerning the Endosperm in Inheritance.

That grain-length is a maternal character was one of the conclusions arrived at by a comparative study of the inheritances of glume-length and grain-length [see § VI (γ), p. 123 above]. Singleness of length-type of all grains borne by "*I*" plants was manifestly a *sine quâ non* of the acceptance of such a conclusion and it was thought well to re-examine the "*I*" grain question both theoretically and in practice.

For the cross here described F_1 data are not available but Biffen [(1) p. 38] found that all the grains borne by all the F_1 (and therefore "*I*") plants were identical in form and appearance and, within the limits of natural fluctuation, of one common length-type. His conclusion is thus precisely the one which has already been expressed in this paper—a conclusion which appears to be decidedly out of harmony with the double fertilization hypothesis.

As already recorded, the F_2 of the Polish × Kubanka cross contained 267 "*I*" plants and their grains afford material for a more extensive inquiry than is possible with the necessarily limited number of F_1 plants in any cross. Table XXV contains the frequency distribution of the lengths of these grains, and it exhibits clear unimodality. That unimodality of distribution is unreliable as an index of singleness of grain-type has, however, already been demonstrated. By measuring the grains of the more numerous $F_3 . I$ plants a distribution based on a greater number of observations might be obtained but it could afford no more evidence as to the existence of sub-types of length than does the $F_2 . I$ distribution. Only by measuring a great number of grains and grouping the lengths of those which grew respectively into *K*, *I*, and *P* plants would it be possible to determine whether the same length of grain (i.e. of endosperm) was or was not associated with the *K*, *I*, and *P* types of embryo. It is proposed to make this test next year but in the meantime, metrical methods failing, the assistance of eye-judgment has been sought. Suitably devised tests demonstrated the ability of the eye to pick out infallibly the members of the three types from an artificially prepared mixture of *K*, *I*, and *P* grains. Beyond this, however, the eye could not go. Despite very extensive trials no evidence of sub-types either for length or appearance could be found among the grains borne by "*I*" plants. If, owing to "double fertilization," the endosperm is "hybrid" in nature, the fact appears to find no expression in the length or appearance of the endosperm both of which are such that they may be described as "maternal" characters.

The expectation derivable from an extension of the double fertilization hypothesis is of interest. Correns (6) and others assume that the secondary or definitive nucleus of the embryo sac exerts twice as much influence on the endosperm as does the single generative nucleus of the pollen grain—the reason for this being that the definitive nucleus is formed by the union of *two* of the polar cells of the original nucleus of the ovule. The male generative nucleus unites, of course, with the female definitive nucleus and from the divisions which follow this union,

the endosperm results. To express the effect of such a "double dose of femaleness":

let p = either a generative nucleus of a pollen grain which carries the "Polish" factor *or* one of the polar cells which unite to form the definitive nucleus of an ovule bearing the "Polish" factor. Let k be similarly used for "Kubanka."

From the gametogenesis of a heterozygous (I) plant the ♂ generative nuclei will be one half (p) and one half (k); the ♀ definitive nuclei will be one half ($2p$) and one half ($2k$). When such a plant is selfed the following endosperms will result:

$$3p, \quad 2p \mp k, \quad 2k \mp p, \quad 3k.$$

Thus four types of endosperm are expected among the grains borne by "I" plants.

An endosperm of constitution $3p$ develops, when nourished by an $F_0 . P$ mother plant, into the characteristic "Polish" form of endosperm; but it may be that when nourished by the less vigorous "I" type of plant it is imperfectly weaned. Consequently its final length may be below the $F_0 . P$ grain-length standard. Similarly $3k$ may be above the $F_0 . K$ standard. The remaining types $2p \mp k$ and $2k \mp p$ will presumably lie between the $F_0 . P$ and $F_0 . K$ types and thus the expectation for the grains borne by "I" plants is four constituents whose length-modes have, owing to irregular development of the endosperms, closed inwards. The intensified overlapping would certainly produce unimodality of the whole distribution.

This theoretical consideration casts no new light upon the problem. As will be observed, it might be elaborated into an explanation of "shift"—an embryo, genetically Polish, fed during development on an "I" plant and nourished during germination by a similarly improperly weaned ($3p$) endosperm, may be expected to grow into a reduced or "shifted" P plant[1]. Several experimental methods of investigating

[1] The probable validity of such an explanation of "shift" is very greatly increased by the interesting facts collected by Kidd & West [(18) especially Part IV, pp. 222—234]. A close —almost linear—relationship is claimed between the amount of food reserve available for the embryo and the adult vigour and ultimate yield of the plant resulting from the seed. If this claim be justified, it is to be expected that in the $P \times K$ cross, there will be a relationship between seed-weight and length of glume of plant resulting from the seed. That $F_2 . K$ glume-length exceeds $F_0 . K$ is consistent with the explanation suggested, for a $3k$ endosperm nourished by an $F_1 .$ "I" mother plant is likely, on an average, to be heavier than if nourished by an $F_0 . K$ mother plant. In the same circumstances an increase in vigour of the embryo accompanying the endosperm is perhaps a legitimate expectation.

this problem are possible but it has been decided to apply them to a cross whose parents are more widely separated in grain-length, -form, and -weight, than are Polish and Kubanka.

XI. Conclusions.

(1) In this cross, length of glume appears to segregate simply and in the F_2 the ratio long : intermediate : short = 1 : 2 : 1.

(2) The "longs" and "shorts" of F_2 although recognisably of the same form as the parents (F_0), yet differ from them in mean glume-length. The average of the longs is reduced by 24·8 % of the F_0 value. In the case of the F_2 shorts there is an increase over the F_0 shorts but it is smaller.

This change or "shift" in the longs of the F_2 is quite definite and cannot be explained by errors of sampling, seasonal variation, or any such cause.

(3) The "shifted" form of longs of F_2 breeds true as far as F_3 (it has not yet been carried further).

(4) When, in the selfing of F_2 heterozygotes, plants of parental type are again evolved, there is no evidence of super-added "shift." Likewise there is no evidence of a tendency to the restoration of F_0 values of mean glume-length, i.e.

$$(F_3 . P \text{ ex } F_2 . I) = (F_3 . P \text{ ex } F_2 . P) = F_2 . P < F_0 . P.$$

(5) For grain-length results are obtained analogous to (1), (2), (3) and (4). The percentage value of the "shift" is, however, less than it is for glume-length, e.g.

$$F_0 . P - F_2 . P = 12{\cdot}5 \text{ \% of } F_0 . P.$$

(6) All the available evidence supports the view that grain-length and glume-length belong to the same generation or, differently expressed, that grain-length is a maternal character. It is difficult to harmonise this view with the double-fertilization hypothesis.

(7) Numerous grain and glume characters appear to be genetic inseparables. Long-type glumes never contain short-type grains and *vice versâ*. Despite the association of glume-length and grain-length, the correlation between these two variables is low. Certain reasons may be urged in explanation.

(8) A development of hairs, far more marked than that exhibited by either parent variety, is found in certain of the F_2 plants. These plants are all of short glume type. Length of glume appears in some manner to inhibit the full development of hairs.

(9) The Inheritance of Hollow and Solid Straw is complicated and a relationship may possibly exist between "shift" in glume-length and nature of straw inheritance.

(10) The investigation is to be continued by growing on the pure F_0 types as well as the "shifted" parental forms from the F_3. By crossing the "shifted" and F_0 types it is hoped to test the behaviour of "shifted" types in inheritance.

Table XVII (p. 131) presents diagrammatically a résumé of the measurable characteristics of the glume types in successive generations.

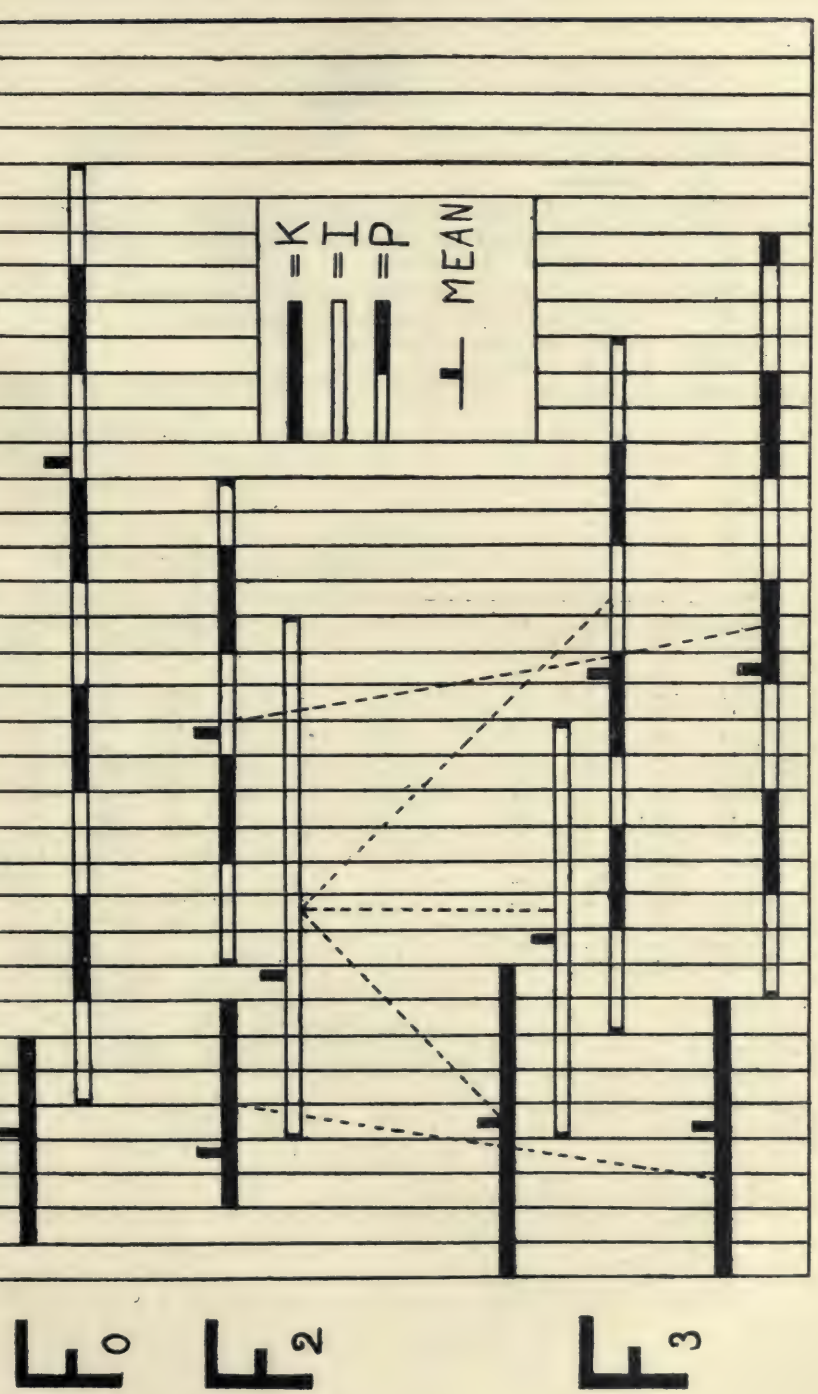
Opportunity is taken here to thank Professor R. H. Biffen, F.R.S., and Mr G. Udny Yule, M.A., C.B.E., for the help they have kindly given during the investigation.

TABLES OF FREQUENCY DISTRIBUTIONS OF GLUME- AND GRAIN-LENGTHS.

Glumes.

Table No.	Type and Generation
I	$F_0 . P$, i.e. Parent variety
II	$F_0 . K$, i.e. Parent variety
III	F_2 complete
IV	The 487 F_2 plants from which seed was obtained for F_3
V	17 F_2 plants which though fertile were unripe at harvest
VI	7 F_2 plants ripe at harvest but sterile
VII	19 F_2 plants unripe at harvest and sterile
VIII	$F_2 . K$, i.e. Kubanka type of the F_2
IX	$F_3 . K$ ex $F_2 . K$, i.e. descendants by selfing of the plants of VIII
X	$F_2 . P$, i.e. Polish type of the F_2
XI	$F_3 . P$ ex $F_2 . P$, i.e. descendants by selfing of the plants of X
XII	$F_2 . I$, i.e. heterozygote (intermediate) plants of F_2
XIII	$F_3 . I$ ex $F_2 . I$, i.e. heterozygous descendants of plants of XII
XIV	Complete descendants of the plants of XII (by selfing)
XV	$F_3 . K$ ex $F_2 . I$, i.e. K type of descendants of the plants of XII
XVI	$F_3 . P$ ex $F_2 . I$, i.e. P type of descendants of the plants of XII
XVII	Diagrammatic résumé of measurable characteristics of K, I, and P in F_0, F_2, and F_3.

TABLE XVII. *A Résumé of the Measurable Characteristics of the K, I, and P Glume Types in F_0, F_2, and F_3.*



Grains.

Table No.	Type and Generation
XVIII	$F_0 . P$, i.e. Parent variety
XIX	$F_0 . K$, i.e. Parent variety
XX	F_2 complete
XXI	$F_2 . K$, i.e. Kubanka type of the F_2
XXII	$F_3 . K$ ex $F_2 . K$, i.e. descendants by selfing of the plants of XXI
XXIII	$F_2 . P$, i.e. Polish type of the F_2
XXIV	$F_3 . P$ ex $F_2 . P$, i.e. descendants by selfing of the plants of XXIII
XXV	$F_2 . I$, i.e. heterozygote (intermediate) plants of F_2
XXVI	Complete descendants of the plants of XXV
XXVII	$F_3 . P$ ex $F_2 . P$ } from separate series of measurements (see § VI of text)
XXVIII	$F_3 . P$ ex $F_2 . I$ }

N.B. Table XXIX is a table of glume-lengths and refers to § VIII of the text.

Grains.

Means of classes in millimetres	Class Means	Numbers of Tables XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII	XXVIII
	5·5	—	2	—	—	2	—	—	—	—	20	4
	6·0	—	—	—	—	5	—	—	—	10	43	19
	6·5	—	9	3	2	17	—	9	—	31	123	53
	7·0	—	47	11	5	56	1	36	5	82	262	94
	7·5	—	111	32	10	169	2	73	16	199	509	207
	8·0	4	187	89	30	399	14	167	45	589	679	324
	8·5	7	36	127	36	347	20	199	68	721	864	321
	9·0	37	2	151	24	189	40	274	87	691	845	343
	9·5	56	1	67	4	25	23	149	40	340	585	199
	10·0	165	—	7	1	5	1	95	5	138	319	76
	10·5	154	—	—	—	—	—	23	—	19	112	4
	11·0	81	—	—	—	—	—	3	—	2	30	—
	11·5	18	—	—	—	—	—	—	—	—	3	—
	12·0	1	—	—	—	—	—	—	—	—	2	—
	Totals	526	395	487	112	1214	101	1028	266	2822	4396	1644

TABLE XXIX.

Class Means	9·5	10·5	11·5	12·5	13·5	14·5	15·5	16·5	17·5	18·5	19·5	Total
Frequencies	4	7	17	2	4	3	5	4	3	4	3	56

Glumes.

Means of classes are in millimetres	Numbers of Tables															
Class Means	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
7·5	—	—	—	—	—	—	—	—	0·5	—	—	—	—	—	1·0	—
8·5	—	3·0	—	—	—	—	—	—	3·0	—	—	—	—	4·5	2·5	—
9·5	—	36·0	17·0	12·5	2·5	—	2·0	12·5	60·5	—	—	—	—	34·0	22·0	—
10·5	—	74·0	31·5	28·0	0·5	1·0	2·0	28·5	221·0	—	—	—	—	115·0	51·0	—
11·5	—	118·5	52·0	47·5	1·5	1·0	2·0	44·0	357·0	—	—	2·0	1·0	217·0	89·0	—
12·5	1·0	105·0	28·5	24·0	2·5	—	2·0	20·0	329·5	—	—	2·5	4·5	202·5	75·0	—
13·5	—	51·5	29·5	25·5	—	—	4·0	6·0	184·0	—	—	19·0	18·5	153·0	36·0	0·5
14·5	—	12·0	44·5	38·5	3·0	—	3·0	1·0	68·5	—	2·5	37·5	42·5	151·0	12·0	1·5
15·5	—	—	47·0	46·0	1·0	—	—	2·0	13·0	—	9·0	44·0	69·0	186·5	2·0	1·0
16·5	—	—	78·0	75·0	1·0	—	2·0	—	—	2·0	9·5	73·0	94·5	238·0	1·0	3·5
17·5	—	—	46·0	43·0	1·0	2·0	—	—	—	2·0	18·5	40·5	110·0	265·0	—	3·0
18·5	—	—	37·0	35·0	1·0	1·0	—	—	—	1·0	31·0	34·0	106·5	246·0	—	6·0
19·5	—	—	15·5	13·5	1·0	—	1·0	—	—	6·5	49·0	7·0	61·0	181·5	—	13·5
20·5	2·0	—	17·0	16·0	1·0	—	—	—	—	11·5	50·0	3·5	44·5	126·0	—	15·5
21·5	0·5	—	13·5	13·5	—	—	—	—	—	12·0	86·5	1·5	18·5	114·5	—	19·5
22·5	6·0	—	16·5	15·5	—	1·0	—	—	—	14·0	101·0	0·5	8·5	79·5	—	26·0
23·5	16·0	—	11·5	10·5	1·0	—	—	—	—	9·0	96·5	1·0	1·0	81·0	—	17·5
24·5	24·0	—	20·0	19·0	—	—	1·0	—	—	20·0	112·5	—	—	79·0	—	34·5
25·5	14·5	—	8·0	7·0	—	1·0	—	—	—	6·0	102·0	—	—	92·5	—	40·0
26·5	34·5	—	10·0	10·0	—	—	—	—	—	9·0	106·0	1·0	—	82·5	—	36·5
27·5	38·5	—	5·0	5·0	—	—	—	—	—	5·0	78·0	—	—	63·5	—	33·0
28·5	39·0	—	1·0	1·0	—	—	—	—	—	1·0	72·0	—	—	51·5	—	19·5
29·5	48·0	—	—	—	—	—	—	—	—	1·0	53·0	—	—	30·0	—	18·0
30·5	66·5	—	1·0	1·0	—	—	—	—	—	1·0	28·0	—	—	23·0	—	13·0
31·5	63·0	—	—	—	—	—	—	—	—	—	18·0	—	—	13·0	—	6·5
32·5	58·0	—	—	—	—	—	—	—	—	—	17·5	—	—	13·0	—	2·0
33·5	69·5	—	—	—	—	—	—	—	—	—	6·0	—	—	6·0	—	4·5
34·5	45·0	—	—	—	—	—	—	—	—	—	3·0	—	—	3·5	—	3·0
35·5	33·0	—	—	—	—	—	—	—	—	—	1·0	—	—	0·5	—	—
36·5	20·0	—	—	—	—	—	—	—	—	—	2·0	—	—	—	—	—
37·5	11·5	—	—	—	—	—	—	—	—	—	0·5	—	—	0·5	—	—
38·5	6·0	—	—	—	—	—	—	—	—	—	—	—	—	0·5	—	—
39·5	2·0	—	—	—	—	—	—	—	—	—	1·0	—	—	—	—	—
40·5	1·0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Totals	600	400	530	487	17	7	19	114	1237	101	1054	267	580	2854	292	318

BIBLIOGRAPHY.

1. Biffen, R. H. "Mendel's Laws of Inheritance and Wheat Breeding." *Journ. Agric. Sci.* Vol. I. 1905—6.
2. Backhouse, W. O. "The Inheritance of Glume-Length in *Triticum polonicum*; A Case of Zygotic Inhibition." *Journ. of Genetics*, Vol. VII. 1917—18.
3. Caporn, A. St Clair. "On a Case of Permanent Variation in the Glume-Length of Extracted Parental Types and the Inheritance of Purple Colour in the Cross *T. polonicum* × *T. Eloboni*." *Journ. of Genetics*, Vol. VII. 1917—18.
4. Collins, G. N. "Correlated Characters in Maize Breeding." *Journ. Agric. Research*, Vol. VI. 1, 1916.
5. Yule, G. Udny. *An Introduction to the Theory of Statistics*, Ed. 1919, p. 303.
6. Correns, C. G. *Biblio. Bot.* LIII. 1—161.
7. Groth, B. H. A. *Bulletins of N. Jersey Ag. Exp. Sta.* Nos. 228, 238, 239, 242, and 278.
8. ——. *Science*, April, 1914.
9. Gates, R. Ruggles. "On the Modification of Characters by Crossing." *Amer. Nat.* Vol. XLIX. 1915.
10. Punnett, R. C., and Bailey, P. G. "On the Inheritance of Weight in Poultry." *Journ. of Genetics*, Vol. IV. 1914—15.
11. —— and the late Major P. G. Bailey, R.F.A. "Genetic Studies in Rabbits. I. On the Inheritance of Weight." *Journ. of Genetics*, Vol. VIII. 1918—19.
12. Castle, W. E., and Phillips, J. C. "Piebald Rats and Selection; an Experimental Test of the Effectiveness of Selection and the Theory of Gametic Purity in Mendelian Crosses." *Carnegie Publ.* No. 195, 1914.
13. Davenport, C. B. "Inheritance in Poultry." *Carnegie Publ.* No. 52, 1906.
14. Leake, H. Martin. "Studies in Indian Cotton." *Journ. of Genetics*, Vol. I. 1911.
15. East, E. M. "Inheritance in Crosses between *Nicotiana Langsdorfii* and *N. alata*." *Genetics*, Vol. I. 1916.
16. Belling, J. "Inheritance of Length of Pod in Certain Crosses." *Journ. Agric. Research*, Vol. V. 1, 1915—16.
17. Balls, W. L. *Studies of Egyptian Cotton*, 1909.
18. Kidd, F., and West, C. "Physiological Pre-determination; the Influence of the Physiological Condition of the Seed upon the Course of Subsequent Growth and upon the Yield." *Ann. App. Biol.* Vol. V. Nos. 3 and 4, April, 1919.

INHERITANCE OF WING COLOUR IN LEPIDOPTERA.

IV. MELANISM IN *BOARMIA ABIETARIA*.

By H. ONSLOW.

(With Plate VI.)

The inheritance of melanism has already been described in two insects closely related to *B. abietaria*, namely *Tephrosia consonaria*[1] and *Boarmia consortaria*[2].

These three insects have scarcely ever been taken north of London, being almost exclusively confined to Kent and Surrey, where another melanic variety, that of *Tephrosia extersaria* (*luridata*), has lately made its appearance. They are thus contrasted with most melanic varieties, which generally come from the Black Country, or other urban districts. A melanic race of *B. consortaria* has, however, recently appeared in Warwickshire.

The melanic variety of *B. abietaria* seems never to have been named, for *sericearia* Curt. is apparently meant to apply to a dark form of the type, somewhat similar to figs. 1 and 2 of Plate VI. There are no records of any extensive breeding experiments, but Bower[3] exhibited a family of 17 insects bred from ova deposited by a type ♀, which had been captured. The ♂ appears to have been a melanic, for 7 of the offspring were melanic and 10 type. Stallman[4] also reared a series of insects bred from a melanic ♀ × a type ♂, which contained no type, but were "all dark to black." These results appear to be in agreement with the conclusion arrived at in this note, namely that the melanic variety behaves as a simple Mendelian dominant to the type form.

[1] Onslow, H., *Journ. of Genetics*, Vol. IX, No. 1, p. 53, Dec. 1919.
[2] Onslow, H., *Journ. of Genetics*, Vol. IX, No. 4, p. 339, Mar. 1920.
[3] Bower, B. A., *Ent. Rec.*, Vol. XI, p. 82, 1899.
[4] Stallman, *Ent. Rec.*, Vol. XXVIII, p. 21, 1916.

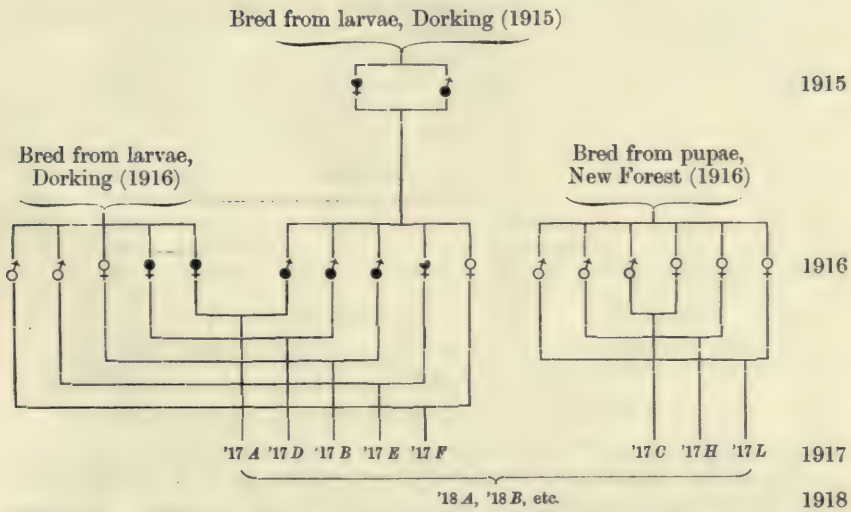
The melanic form of *B. abietaria*, though very dark, is not so sharply differentiated from the type as is sometimes the case This is partly because the shade of the type form varies considerably, but chiefly because it is by nature a dark species. Plate VI, figs. 1 and 2, show the darkest specimen of the type insect met with, which is almost certainly identical with var. *sericearia* Curt. This variety only occurred occasionally, and was usually extracted from melanics. It is so dark that it shows traces of black veining like the true melanic form, and the casual observer might possibly mistake it for that variety. The difference is, however, manifest, for the whole insect is browner than the black form, and appears slightly mottled especially on the hind wings. The three black spots on the costa can be always faintly discerned, and the deep black lunules along the margin are also visible. These lunules are especially conspicuous on the hind wing where they form the most distinctive feature, by which the type insects can be characterised. In the melanic form (figs. 9 and 10) the wings of the female are a uniform velvety black, those of the male slightly browner, and except the discal streak and the veins which are traced in deeper black there are no lines or markings of any description. This black veining is especially evident in insects which are freshly emerged, but it fades on drying. The type strain with which the melanics were crossed, came from pupae collected in two separate districts in the New Forest. They varied in colour and markings, sometimes being as dark as insect No. 3, which was extracted from melanics, but the majority were like insects Nos. 5 and 6. In two cases wild insects emerged as pale as No. 8, and were a lovely golden brown. The colours seem to get progressively less golden and more grey, as the insect gets darker. No. 7 is one of a long series of typical insects, bred by Mr Harwood from New Forest pupae, the colour of which was almost uniformly like that of the specimen illustrated. On the whole, the females as a rule appear slightly darker than the males.

The melanic strain came from two batches of larvae collected from the yew trees at Dorking, one in 1915 and the other in 1916; in the following year these produced families '17 *A*, '17 *B*, etc. as is seen from the accompanying table. Each family is labelled with a distinctive letter and the year in which the ova were laid.

The larvae proved very hardy, especially the melanic race. The ova were hatched in metal boxes, and were transferred a fortnight later to yew in the ordinary type of glass breeding cylinders, where they remained until ready to hibernate. During the winter, they were sleeved on yew

GENEALOGICAL TABLE

of *B. abietaria*.



trees, the bark of which they are accustomed to eat until the spring. As soon as the oak was out, they were returned to the cylinders, where they were fed until they pupated, as oak was easier to obtain in large quantities than yew. From the twenty pairings made in 1918, over 1600 insects emerged in the following year.

The melanic form, as will be seen from the following experiments, clearly behaves as a simple Mendelian dominant, no matter how dark the type may be. The result of pairing two melanics, one at least of which was homozygous, was as follows:

Melanic × *Melanic*.

[*DD* × *DD* (*DR*)].

	Imagines					
	Melanic			Type		
Family	Male	Female	Totals	Male	Female	Totals
'17 *A*	21	13	34	—	—	—
'17 *D*	7	7	14	—	—	—
'18 *A*	13	8	21	—	—	—
'18 *B*	85	112	197	—	—	—
'18 *E*	72	87	159	—	—	—
'18 *F*	5	11	16	—	2	2
'18 *T*	19	19	38	—	—	—
	Totals ...		479			2

The two type females which are included represent less than 0·5 per cent., and are no doubt due to the accidental inclusion of eggs or larvae with the wrong brood.

The result of pairing two melanics, both of which were heterozygous, was as follows:

Melanic × *Melanic.*

[*DR* × *DR*].

	Imagines					
	Melanic			Type		
Family	Male	Female	Totals	Male	Female	Totals
'18 *C*	31	24	55	10	5	15
'18 *D*	14	16	30	4	7	11
Totals			85 (77 °/₀)			26 (23 °/₀)
Expectation ...			*83*			*28*

The expected ratio is very nearly realised, in spite of the fact that there were only two pairings of this nature. There is as usual an excess of a few melanics. A few of the extracted types were as dark as var. *sericearia* (Pl. VI, figs. 1 and 2), and most of them were rather darker than the wild types, though a few resembled figs. 5 and 6.

The result of pairing homozygous melanics by wild types from the New Forest strain, was as follows:

Melanic × *Type.*

[*DD* × *RR*].

	Parentage		Imagines					
			Melanic			Type		
Family	Female	Male	Male	Female	Totals	Male	Female	Totals
'18 *O*	Melanic	× Type	33	29	62	—	—	—
'18 *P*	Type	× Melanic	41	32	73	—	—	—
'18 *X*	Type	× Melanic	13	19	32	—	—	—
Total					167			

In this case the offspring include no type specimens, but only melanics, as was expected.

The result of pairing heterozygous melanics by type was as follows:

Melanic × *Type.*

[*DR* × *RR*].

	Parentage		Imagines: Melanic			Imagines: Type		
Family	Female	Male	Male	Female	Totals	Male	Female	Totals
'17 *B*	Type	× Melanic	8	5	13	4	8	12
'17 *E*	Melanic	× Type	11	7	18	1	4	5
'17 *G*	Type	× Melanic	11	10	21	16	11	27
'18 *G*	Type	× Melanic	23	22	45	26	26	52
'18 *H*	Melanic	× Type	18	21	39	17	10	27
'18 *N*	Melanic	× Type	13	11	24	12	10	22
'18 *Q*	Melanic	× Type	39	16	55	34	22	56
'18 *W*	Type	× Melanic	9	4	13	9	6	15
		Totals			228 (51 %)			216 (49 %)
		Expectation ...			*222*			*222*

The actual ratio of type to melanic in the above crosses is very close to equality, there being a slight excess of melanics.

The type parents of families '17 *B*, '17 *E* and '17 *G*, as may be seen from the genealogical table, all come from the Dorking strain. In the following year the type parent of '18 *G* is extracted from melanics, but in the other families the type parents come from the wild New Forest strains. The ♂ parent of '18 *Q* was exceptionally pale, and is shown on Pl. VI, fig. 8. All the type offspring of this family were appreciably lighter than those from others; and they had a much richer and somewhat golden colour. Not one of them, however, was as pale as their father.

A few pairings were also made by mating together types obtained in different ways, and the results were as follows:

Type × *Type.*

[*RR* × *RR*].

	Parentage		Imagines: Melanic			Imagines: Type		
Family	Female	Male	Male	Female	Totals	Male	Female	Totals
'17 *C*	T. (New Forest)	× T. (New Forest)	—	—	—	5	4	9
'17 *F*	T. (from melanics)	× T. (Dorking)	—	—	—	18	16	34
'17 *H*	T. (New Forest)	× T. (New Forest)	—	—	—	16	9	25
'17 *L*	T. (New Forest)	× T. (New Forest)	—	—	—	13	9	22
'17 *M*	T. (New Forest)	× T. (New Forest)	—	—	—	17	6	23
'18 *I*	T. (from melanics)	× T. (New Forest)	—	—	—	33	25	58
'18 *J*	T. (from melanics)	× T. (from melanics)	—	—	—	94	78	172
'18 *K*	T. (from melanics)	× T. (from melanics)	—	—	—	39	40	79
'18 *L*	T. (from melanics)	× T. (from melanics)	1	1	2	47	45	92
'18 *M*	T. (from melanics)	× T. (from melanics)	—	—	—	34	31	65
		Totals ...	...	...	2			579

Out of the large number of type insects bred, only 2 proved melanic, that is less than 0·5 per cent., and these were without doubt introduced accidentally. In families '17 *C*, *H*, *L* and *M* both parents were wild types from the New Forest. In family '17 *F* the ♀ parent was extracted from melanics, and the ♂ came from the Dorking strain. In family '18 *I* the ♀ parent was extracted from melanics, and the ♂ came from the New Forest. In families '18 *J*, *K*, *L* and *M*, both type parents were extracted from melanics. It is thus evident that the black form of *B. abietaria*, like the other closely related species investigated, behaves as a simple Mendelian dominant. Though the type is subject to a great deal of variation, no distinct inheritance of an intermediate variety, such as var. *sericearia*, could be detected.

My thanks are due to Prof. Punnett and to the late Prof. Doncaster for kindly reading the MS. of this paper, and to Miss Helen Moodie for her care of the larvae, and the large broods reared.

DESCRIPTION OF PLATE VI.

Boarmia abietaria and var. *sericearia*. Natural size.

1. Var. *sericearia* ♀. Bred from melanics.
2. Var. *sericearia* ♂. Bred from melanics.
3. *B. abietaria* ♀. Dark type, bred from melanics.
4. *B. abietaria* ♂. Dark type, bred from New Forest strain.
5. *B. abietaria* ♀. Type, bred from New Forest strain.
6. *B. abietaria* ♂. Type (New Forest).
7. *B. abietaria* ♀. Type (New Forest) from Mr Harwood.
8. *B. abietaria* ♂. Type, golden-brown (New Forest).
9. *B. abietaria* ♀. Melanic, bred from Dorking strain.
10. *B. abietaria* ♂. Melanic, bred from Dorking strain.

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THE INHERITANCE OF SPECIFIC ISO-AGGLUTININS IN HUMAN BLOOD.

By J. R. LEARMONTH.

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Introduction.

The present enquiry arose out of work upon the clinical aspects of transfusion of blood. In the course of this work, it became apparent that it would be interesting to ascertain if the incidence of specific iso-agglutinins in the blood, with the corresponding agglutinophilic reactions of the red cells, obeyed in man the biological laws of Mendelian inheritance. No previous work upon this subject including a survey of families could be found though a Mendelian interpretation has been recently suggested by Hirschfeld and Hirschfeld (1919). In the following report, a series of observations upon forty families will be discussed on these lines.

History.

In 1901, Landsteiner definitely showed that iso-agglutination reactions in human blood were not confined to individuals suffering from disease, but that these reactions were also produced upon mixing the bloods of normal people. This investigator classified human sera into three groups, according to their iso-agglutinating action. Moss, in 1910, as the result of further investigations, was able to add to these a fourth group, and his classification of human sera is shown below in tabular form.

Group	Red corpuscles agglutinated by sera of groups	Serum agglutinates R. B. C. of groups
I	II, III, IV	—
II	III, IV	I, III
III	II, IV	I, II
IV	—	I, II, III

The work of Moss has proved of great importance as a necessary preliminary to blood transfusion, especially in the War. Prior to his introduction of the group-compatibility tests, severe reactions after transfusion were by no means uncommon.

Theoretical Considerations.

The division of human sera into four groups, in the manner suggested by Moss, has tended to some degree to obscure the problems connected with the inheritance of those qualities upon which the classification is based. Taking as the precise quality the agglutinophilic reactions of the red cells of the blood, inspection of the table above reveals the fact that there are but two such characteristics fundamentally present in human blood—namely those possessed by individuals of Groups II and III. Group I consists of individuals in whose red cells both these characteristics are found; while in the red cells of individuals belonging to Group IV neither characteristic is present. Similarly, the iso-agglutinating power of a Group IV serum is built up from the presence, and that of a Group I serum from the absence, of both the iso-agglutinating capacities which separately characterise the sera of Groups II and III. That the individual agglutinophilic capacities of Group II cells and Group III cells are mutually exclusive, i.e. that one such characteristic cannot be present at the same time as the other, is precluded by the existence in Group I of cells which display both capacities towards appropriate agglutinating sera. Similarly, the agglutinating capacity of Group IV serum affords proof of a similar condition of affairs with regard to the iso-agglutinins found in Groups II and III respectively. The work of Landsteiner makes it abundantly clear that the agglutinating power of the serum of a blood, and the agglutinophilic capacity of its red cells, are reciprocal in terms of the aforesaid characteristics. That is, a Group II blood, in which the red cells are agglutinable by a Group III serum, has in its own serum an agglutinin for the cells of a Group III blood; and *vice versa*. So also Group I blood contains no iso-agglutinin in its serum; and a Group IV blood contains iso-agglutinins for the red cells of Groups I, II, III.

Von Dungern and Hirschfeld (1911) give the symbol α to the iso-agglutinophilic capacity displayed by Group II red cells, and β to that displayed by Group III red cells. These symbols define the specificity both of the agglutinophilic capacity of the red cells, and of the iso-agglutinin in the serum, if cognisance be taken of the work of Landsteiner referred to above. The cells of Group IV, which show no iso-agglutinophilic capacity, they consider to carry properties which they name non-α and non-β. To bring this terminology into line with that generally adopted in genetics we may regard the agglutinophilic capacity as due to definite

factors—that displayed by Group II to factor **A**, and that by Group III to factor **B**. The corresponding small letter serves to signify the absence of either factor. **A** therefore corresponds to the α of Von Dungern and Hirschfeld, **B** to the β; while the conditions non-α and non-β of these authors are represented respectively by **a** and **b**.

TECHNIQUE.

1. *The provision of test sera.* In order to assign a particular blood to one of Moss's four Groups, it is unnecessary to have specimens of the serum of each Group. It will be seen from the table on page 141 that a supply of only two sera is essential, viz. those of Groups II and III. The method of determination is shown in the following table (after Moss):

Unknown blood	Reaction of R. B. C. of unknown to Group II serum	Group III serum	Group of unknown
X	+	+	I
X	0	+	II
X	+	0	III
X	0	0	IV

+ = agglutination.
0 = no agglutination.

It therefore facilitated the work to have available at its commencement a supply of the sera of Groups II and III from an authentic source.

Since additional supplies of the test sera become necessary, the manner of procuring, selecting, and storing these must be briefly considered.

It is a matter of experience that sera which belong to the same group vary within wide limits in their agglutinating capacities; and further that the agglutinophilic capacity of the red cells of a given blood, or, in the case of Group IV, of its absence, is no index of the potency of its agglutinin content. In order to obtain the most active Group II and Group III sera, small quantities of the sera of individuals of these groups are tested against washed red cells of individuals belonging to Group III and Group II respectively. Approximately equal quantities of serum and cells are mixed in each case under similar conditions of temperature etc., and those sera which soonest cause agglutination of the washed cells are selected for stock.

A suitable donor having been found, about 20 cc. of blood are withdrawn with aseptic precautions from a superficial vein of the arm into a sterile receptacle. The blood is allowed to clot, centrifuged in sterile tubes, and the serum pipetted off in quantities of about 5 cc. into rigidly sterilised glass tubes, which are then sealed in the blowpipe, labelled with the

group of the serum, and stored in the dark. The action of light is said to hasten the disappearance of the iso-agglutinin, which otherwise occurs very slowly; and serum prepared and kept in this way remains active, in the writer's experience, for at least three months.

When required for use the serum is transferred to sterile bottles, of the type known to dispensing chemists as "nitric acid bottles"; the wooden cases provided with these protecting the serum from the action of light. To each 5 cc. of the serum is added, as a preservative, three drops of a 0·1 °/₀ solution of proflavine in physiological sodium chloride. Flavine and its derivatives normally agglutinate washed red cells, but this effect is absent when serum is present with the corpuscles. It is found that proflavine in the proportion stated prevents the growth of bacteria during the period the serum is in constant use, provided care be taken to introduce only sterile appliances into the bottles. The disadvantage of chloroform in small quantities as a preservative is that when the cells to be tested are of weak agglutinophilic capacity, lysis may precede and interfere with the observation of agglutination (cf. Robertson, 1918).

2. *The performance of the test.* A spirit lamp should be always carried to cleanse the platinum loop with which the preparations are made. Two clean slides, labelled 2 and 3 respectively, are used for each test. Upon each is placed two loopfuls of the appropriate serum, care being taken to cleanse the loop carefully in the flame before introducing into the second bottle. The tip of a finger is cleansed with alcohol, and pricked with a sterile needle. To the serum on each slide is added a loopful of blood, and the preparation thoroughly mixed with the loop; care being taken as before to cleanse the wire in the flame between contact with different sera. By rocking the slides to and fro, agglutination if present is usually visible in a minute or two at room temperature. Doubtful cases may be gently warmed, and rocked for ten minutes before readings are taken.

Two points of some practical importance may be here commented upon. The use of hollow ground slides when performing the test in this manner is to be avoided, since the red corpuscles may sink to the bottom of the depression, and simulate a massive agglutination where none is present. This is particularly liable to take place when the slide is insufficiently rocked.

Further, in about 25 °/₀ of cases iso-haemolysin is found in a given human blood serum in addition to iso-agglutinin. This factor however does not interfere with the test, since, as Moss puts it, "all sera contain an anti-haemolysin which protects the homologous corpuscles and those

of the same group from solution by any other serum." It is also found that agglutination always precedes haemolysis.

3. *Recording of results.* This is done upon slips prepared as shown below.

Index No. of Family .....
Father: Group .....
Mother: „ .....
C: „ .....
C: „ .....
C: „ .....
C: „ .....
C: „ .....
C: „ .....

M = Male, F = Female, child.

STATISTICS.

The data given in Table I (p. 146) are compiled from a series of forty families, taken at random.

ANALYSIS OF FAMILIES.

To facilitate an analysis of these 40 families they have been arranged under the ten possible forms of mating in Table II (p. 147). Three of these forms, viz. I × I, I × III, and III × III are not represented in the data. Since there is no indication of sex-linked inheritance no distinction has been made between the sexes of the offspring in Table II, but their total numbers have been allocated to the respective groups to which they belong. Reciprocal matings also are here grouped together.

With a single exception, noted below, the facts are clearly in accordance with the explanation already outlined in terms of the two factors **A** and **B**. The following comments on the Table may be given in connection with certain of the types of mating.

(*b*) This form of mating might give rise to many different results according as to whether the Group I parent were of the constitution **AABB**, **AaBB**, **AABb**, or **AaBb**, and the Group II parent **AAbb** or **Aabb**. Theoretically offspring of all four classes could arise from this form of mating if the parents were **AaBb** and **Aabb**. This of course also is true of (*c*).

(*d*) The absence of offspring other than of Group I suggests that the parents of this form were likely to have been of the constitution **AABB**.

(*e*) This form of mating should give either offspring of Group II only, or else of Group II and Group IV in the ratio 3 : 1. In one family only did a recessive individual of Group IV appear. The numbers in the families are however small, and it is possible that in other instances the matings may have been between heterozygotes.

(*f*) This mating should bring together the two factors **A** and **B**, and lead to the production of some offspring of Group I. In the single family recorded such was the case.

TABLE I.

Index number of family	Father	Mother	Children	
			Males	Females
1	4	4	—	4
2	2	2	2	2
3	4	4	4	—
4	4	2	—	2, 2
5	2	2	—	2, 4
6	2	2	2, 2	—
7	4	4	4, 4	4
8	2	4	2	—
9	2	1	1	2, 1
10	4	4	4, 4, 4,	4
11	4	2	4, 2	4, 4, 2, 2, 2
12	4	4	—	2, 4
13	4	4	—	4, 4
14	4	2	2, 4	2, 2, 4
15	2	2	—	2, 2
16	2	4	—	4, 2, 2, 2
17	2	4	2	2, 2
18	2	1	1	—
19	2	4	2, 2	2, 2
20	4	3	—	4, 4
21	3	4	3	3, 3
22	2	4	2	—
23	4	1	1	1
24	2	4	2	—
25	2	4	—	2, 2
26	2	2	2	—
27	4	2	4, 2	2
28	2	1	2, 1	—
29	4	1	1, 1, 1, 1	1, 1
30	2	2	2, 2	—
31	4	4	4, 4	—
32	2	4	—	2, 2
33	4	4	—	4, 4
34	4	2	2	2, 4
35	4	3	—	3, 4
36	4	4	—	4, 4
37	4	2	2, 2	—
38	4	1	—	1, 1, 1, 1
39	2	3	—	1, 1, 3
40	2	2	2	—

TABLE II.

Type of mating	No. of family	No. of offspring in each of the four groups I	II	III	IV
(*a*) I × I (no example)	—	—	—	—	—
(*b*) I × II (3 families)	9	2	1	—	—
	18	1	—	—	—
	28	1	1	—	—
(*c*) I × III (no example)	—	—	—	—	—
(*d*) I × IV (3 families)	23	2	—	—	—
	29	6	—	—	—
	38	4	—	—	—
(*e*) II × II (7 families)	2	—	2	—	—
	5	—	1	—	1
	6	—	2	—	—
	15	—	2	—	—
	26	—	1	—	—
	30	—	2	—	—
	40	—	1	—	—
(*f*) II × III (1 family)	39	2	—	1	—
(*g*) II × IV (14 families)	4	—	2	—	—
	8	—	1	—	—
	11	—	4	—	3
	14	—	3	—	2
	16	—	3	—	1
	17	—	3	—	—
(*g*) II × IV (14 families)	19	—	4	—	—
	22	—	1	—	—
	24	—	1	—	—
	25	—	2	—	—
	27	—	2	—	1
	32	—	2	—	—
	34	—	2	—	1
	37	—	2	—	—
(*h*) III × III (no example)	—	—	—	—	—
(*i*) III × IV (3 families)	20	—	—	—	2
	21	—	—	3	—
	35	—	—	1	2
(*j*) IV × IV (9 families)	1	—	—	—	1
	3	—	—	—	1
	7	—	—	—	3
	10	—	—	—	4
	12	—	1	—	1
	13	—	—	—	2
	31	—	—	—	2
	33	—	—	—	2
	36	—	—	—	2

(*g*) In this, the largest group of families, there are evidently two types of mating, viz. **Aabb** × **aabb** and **AAbb** × **aabb**. In Nos. 11, 14, 16, 27, 34 the Group II parent was evidently heterozygous, and the offspring of the 5 families together comprise 14 dominants and 8 recessives where equality was to be expected. In the remaining families the Group II parent may have been of the constitution **AAbb**.

(*i*) The data are scanty but suggest that individuals of Group III are more often heterozygous than those of Group II. This is in accordance with the greater scarcity of Group III individuals in an English population (cf. Hirschfeld and Hirschfeld, 1919).

(*j*) Here we should expect nothing but Group IV individuals. A single exception in the form of a Group II individual occurred in Family No. 12. Hirschfeld and Hirschfeld (1919) have recently suggested that in such cases the putative father is not the real father. They are inclined to regard the appearance of a child belonging to Groups I, II, or III from parents belonging to Group IV as a proof of illegitimacy, and point out that such occurrences may afford a valuable medico-legal test.

Conclusion.

From the foregoing considerations it is permissible to deduce that the iso-agglutinins present in human blood are inherited, and that as a general rule the inheritance follows Mendelian laws. There arises however a modifying factor, in the observation by Bond, that iso-agglutinins formerly absent in the serum of a given person may appear in that serum after the person has successfully withstood a systemic infection. It would be interesting in such cases to know the groups to which the parents of the person belonged.

The writer gladly takes this opportunity of recording his indebtedness to Prof. D. Noël Paton, in whose laboratory the work was carried out, for assistance in its inception, and help in its execution; to the Medical Research Committee for the monetary grant which made the work possible; to Dr J. W. McNee, D.S.O., Prof. W. Bulloch, Col. C. J. Bond, C.M.G., for supplying references and hints on technique; to Mr J. M. Graham, F.R.C.S.E., for the original supply of test sera; and to Prof. Punnett, who has kindly revised the section "Analysis of Families."

REFERENCES.

Bond, C. J. *Brit. Med. Journ.* December 8, 1917.
—— *Brit. Med. Journ.* March 2, 1918.
Hirschfeld, L. and Hirschfeld, H. *Lancet*, October 18, 1919.
Landsteiner, W. *Wien. Klin. Woch.* No. 14, p. 1132, 1901.
Moss, W. L. *Bull. Johns Hopkins Hosp.* p. 63, March, 1910.
—— *Journ. Amer. Med. Ass.* June 23, 1917.
Robertson, O. H. *Memorandum on Blood Transfusion, M.R.C.* Sept. 1918.
Von Dungern and Hirschfeld, L. *Zeit. f. Immunitätsforsch.* No. 8, p. 526, 1911.

MULTIPLE ALLELOMORPHS AND LIMITING FACTORS IN INHERITANCE IN THE STOCK (*MATTHIOLA INCANA*).

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(With Plates VII and VIII and 3 Text-figures.)

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Introduction.

Two forms of the Garden Stock are commonly recognised and are familiar to every horticulturist. In respect of the one external character in which they differ, viz. hair-production, they show the extreme limit of range in either direction. At the topmost level stands the type exhibiting a maximum degree of hoariness in all aerial green structures and a sprinkling of hairs even on the petals: at zero point the so-called wallflower-leaved or glabrous variety, which in its extreme form lacks even a single hair. Almost unknown however is the interesting and rare intermediate described in the earlier accounts as half-hoary[1]. This latter plant was originally obtained, together with the

[1] *Reports to the Evolution Committee, Roy. Soc. Report I*, p. 33, 1902, and *Journal of Genetics*, Vol. v. No. 3, p. 145, 1916.

white-flowered form of *incana*, from a coastguard station in the Isle of Wight, but I have never again met with it in cultivation nor been able to obtain it through commercial sources except by accident. As I have previously stated[1] it is without doubt the unnamed variety described by Linnaeus as "*alba nuda vixque tomentosa*[2]." Proved now to be of constant appearance and to breed true it becomes desirable to give it a varietal title, and I propose therefore to name it var. *semi-incana*. Some account of the relations of this little known form both to the type and to the glabrous variety has already been given[3], but several points still required investigation. With the further evidence now before us we are able to complete the account of the inter-relations of these three forms. Furthermore we gain from the new facts additional insight into the mode of action of the so-called "factors" of Mendelian theory. The trend of the new evidence is strongly towards an interpretation of the factorial scheme, in the present case, in terms of physiological states: towards the conception that a "factor" represents something beyond the mere existence of some definite chemical component—that it stands for something in the nature of a particular condition of physiological equilibrium. A more detailed consideration of this aspect of the subject, however, will be better postponed until we have dealt with the whole body of facts now available.

From the earlier work it was already apparent that although the half-hoary variety is intermediate in character between the two recognised forms on the market, it is not to be obtained by crossing these forms together. It was clear that it must contain some factor which is not present in either, or some combination of factors which is not realised by their union. Matings of *semi-incana* with the fully hoary type showed full hoariness to be dominant, but a cross with one of the several glabrous (wallflower-leaved) strains led to the appearance of an entirely new grade—the quarter-hoary, a form intermediate again between the half-hoary and the glabrous condition[4]. The behaviour of this new form proved that quarter-hoariness was invariably associated with a heterozygous constitution. The quarter-hoary grade cannot be fixed as a true-breeding form as can the fully hoary, the half-hoary and the several glabrous strains, for the quarter-hoary individual produces no gametes of corresponding character.

[1] *Journal of Genetics*, *loc. cit.*
[2] *Species Plantarum*, 2nd ed. p. 925, 1762.
[3] *Journal of Genetics*, *loc. cit.*
[4] *Journal of Genetics*, *loc. cit.* p. 152.

By intercrossing therefore the gap between the half-hoary form, itself an intermediate, and the glabrous type was partly bridged, and we had the now expanded series fully hoary, half-hoary, quarter-hoary, glabrous, of which both intermediate grades were characterised by the presence of a new factor not met with in either of the extreme types. In the light of the indications afforded by these results as to the nature of the relation of this new factor to some components of the 4-factor[1] group conditioning full hoariness, it was tempting to hazard a forecast as to the expectation in cases of union with other components of this group, assuming the simplest kind of inter-relation uncomplicated by disturbing causes such as coupling, repulsion and the like. It was not overlooked however that this new factor, provisionally regarded in the absence of definite evidence pointing to a different view as a new and distinct determiner for surface character, and hence denoted by a separate symbol, might prove on fuller investigation to yield results showing that it should more justly be regarded as a modified (? weakened) variant of one or other of the two original determiners for hoariness, rather than as a third factor distinct from either. That we must accept this latter view as the correct one has now become clear, the later work having shown that one of the two determiners for full hoariness must be held to stand in a special allelomorphic relation to its substitute in the intermediate grades. It follows that the prediction based on the assumption of a simple Mendelian relationship involving *three distinct* factors is not realised in those unions in which this multiple allelomorphism comes into play, and that the theoretical ratios tentatively put forward in the earlier account require modification in accordance with this new fact[2]. Furthermore we find, as was not unsuspected in view of the allelomorphic relation between the special factor peculiar to the partially hoary forms and the corresponding factor for full hoariness, that partial hoariness, like full hoariness, is only manifested when the individual contains in addition the two factors for sap colour[3], a point which the earlier experiments had left undecided. It can now in fact be shown that the colour couple acts as a limiting factor for hoariness in all grades included in the hoary group. In the absence of either or both colour components the most that we find is an occasional

[1] Consisting of the two pairs *HK* and *CR*, of which the former (*HK*) has been shown to stand in a causative, the latter (*CR*) in a permissive, relation to the manifestation of hoariness.

[2] See *Journal of Genetics*, *loc. cit.* p. 157.

[3] I.e. the permissive pair referred to above in footnote 1.

hair on the petiole margin of some of the larger leaves. (See later p. 157 where the sub-glabrous forms are described in detail.) So far the partially hoary forms have not been obtained in combination with the full sap-colours. It would seem that either the factors which bring about a diminution in depth of colour and degree of hairiness must be closely coupled, or the same factors must be concerned in producing the dilution effect in both cases. Beyond this fact however we have at present no knowledge of the nature of the bond linking together sap-colour and surface character, and further consideration of this point must be left to the future.

A further important result of the crossbreeding work is the appearance of yet another new grade occupying a position in the series between the half-hoary and the quarter-hoary forms, and hence conveniently termed the mid-hoary grade. Like the quarter-hoary the mid-hoary form is always heterozygous in constitution. These two crossbreds complete the bridging of the gap, already partly filled by *semi-incana*, between the condition of maximum effect seen in the fully hoary individual and the stages leading to the complete disappearance of the hairs in the lowest (= absolutely glabrous) grade. In these last stages preceding final disappearance (i.e. in the sub-glabrous grades) the very slight degree of hairiness developed owing to the presence of *one only* of the two factors causing hair-production is manifested in localised regions of the plant, differing according to which member of the pair is present. Hence it is possible, even in these lower grades, by close inspection, to obtain some information as to their factorial composition, and to dispense with breeding tests.

It is thus apparent that the two familiar garden types—the fully hoary and the wallflower-leaved—are by no means such isolated forms as they have hitherto appeared to be, but represent the end terms in a graded series, each member of which has a distinct appearance and behaviour corresponding to its factorial composition. As to the order in which the varieties *glabra* and *semi-incana* appeared the historical records make no definite pronouncement. Reference to the smooth form can be traced indeed almost as far back as the earliest record known to us of the double-flowered plant (probably 1568)[1]. Both are mentioned by Parkinson in 1629. In an introductory chapter of the *Paradisus terrestris* on "the Ordering of the Pleasure Garden" he writes "Stock-Gilloflowers likewise are almost as common as Wall-flowers, especially the single kindes in every womans Garden, but the double

[1] See Dodoens, *Florum et coronariarum*.....

kindes are much more rare, and possessed but of a few[1]." Later we find enumerated the several kinds and among them the single pale yellow Stocke-Gilloflower (*Leucoium sativum albido luteum simplex*) with the following comment—"There is very little difference in this kind from the former, for the manner of growing, or forme of leaves or flower. Only this hath greener leaves, and pale yellow almost white flowers, in all other things alike[2]." This latter plant will probably have been similar to the wallflower-leaved cream known to-day as Princess May. Still tracing backwards we find in the works of C. Bauhin[3] (1620) and Camerarius[4] (1588) reference to the existence of a plant included by each writer in the section treating of the Wallflower and the Stock, and described generally as white-flowered, sweet-smelling, with green leaves resembling those of the Wallflower rather than the Stock, a sufficient indication that the plant in question was in fact a wallflower-leaved Stock. At this point however we lose track of our plant and its earlier history remains untold. *Semi-incana*, on the other hand, does not appear to have been recorded until about two hundred years later. Linnaeus makes no mention of it in the first edition of the *Species Plantarum* (1753) but includes it in his second edition (1762)[5]. It is thus clear that a glabrous form was at all events well known long before the half-hoary variety was recognised and described. It can therefore hardly be doubted that the larger mutation appeared first. This is quite in accord with the view taken in numerous other cases where the difference between the forms in a series is, as here, one of degree.

We may now set out in fuller detail the results of the later work and the conclusions to which they lead. It will conduce to clearness if as a preliminary we briefly restate the relations which the earlier experiments had shown to hold good, and which in the present account we are able to carry further. These may be summarised as follows:

[1] P. 11. [2] P. 260.

[3] *Prodromus*, p. 102.

[4] *Hortus Medicus*.

[5] P. 925. This early description appears a few years later (*Mantissa* II, p. 428, 1767) in amplified form thus—*flore albo foliis minus tomentosis et viridibus sed non, ut Cheiri, rigidulis*. It will be observed that in each case the plant is described as white-flowered. This was also the case with my own original strain raised from the Isle of Wight seed, but the commercial material which came into my hands later proved to be a coloured race. Absence of sap colour in this case is not however due to absence of the *CR* couple for both these factors have been proved to be present, though for some cause not yet clearly understood they are ineffective. Hence, like *incana* v. *alba* and the white-flowered hoary Brompton strain, white *semi-incana* is to be classed as an inhibition (as opposed to a defective) white. (See *Journal of Genetics*, *loc. cit.* p. 147, footnote 1.)

Summary of Earlier Results.

1. The fully hoary condition is dominant to glabrousness.

2. Full hoariness is primarily dependent upon the presence of a pair of factors denoted by *H* and *K*.

3. The *HK* couple is only fully effective in those individuals which contain in addition another pair of factors, *C* and *R*, upon the presence of which the appearance of anthocyanin in the petals depends. In other words the *CR* couple may be looked upon as constituting a limiting factor conditioning the manifestation of the *HK* effect (full hoariness).

4. All four factors segregate separately, hence every factorial combination can be obtained by suitable matings.

5. Individuals containing all four factors, whether homozygous or heterozygous in respect of any or all, are fully hoary, in other words only one dose of each factor is required to produce full hoariness.

6. Partial hoariness results, when *H* is replaced in the *CRHK* group by another factor indicated in the earlier accounts by *J*.

7. An individual homozygous for all the four factors *CRJK* exhibits the condition described as half-hoary. [That only one dose of *CR* is however *essential*, both here and in the following case, will appear later.]

8. If the half-hoary form is crossed with a glabrous individual of the composition *CRK* the resulting heterozygote is of the lower intermediate grade termed quarter-hoary.

The above inter-relations having been established there remained to be investigated the appearance and behaviour of individuals containing the various other possible combinations of *C*, *R*, *H* and *K* with *J*. The further study which this work has entailed has not only resulted in an addition of new forms to our list of biotypes, but has enabled us to carry our factorial analysis to a further point than had hitherto been achieved by inspection alone. Identification by this means is in some cases however decidedly laborious, necessitating close and repeated examination at different stages of development.

Description of Biotypes.

In the first place we may draw attention to certain anatomical features common to all grades. A noticeable thickening of tissue occurs in every case at the extremity of the leaf over the termination of the midrib. This region shows the structure of a typical watergland

(hydathode). The vascular elements end in a mass of closely packed cells devoid of chlorophyll and with conspicuous nuclei. In most leaves this terminal gland is the only one present. Exceptionally, where one or two strong lateral veins occur, they also terminate in a hydathode giving rise to a well-marked lateral tooth. In the epidermis covering the glands, as elsewhere, stomata of varying size and in all stages of development are abundant. The intervening cells here and over the whole leaf surface vary considerably in size. Those which grow out into hairs show a bulbous basal region and an upper free portion characteristically branched. The walls are cuticularised and very much thickened. The thickening takes place not only in the whole extent of the exposed wall but in the lower sunk portion as well. It may even involve the outer wall of neighbouring cells though these cells do not themselves grow out into hairs. In a surface view of the epidermis the somewhat small bulbous base forms a centre round which the larger neighbouring cells become arranged, more regularly than elsewhere, as a rosette. It is somewhat interesting that although stomata are apparently formed continuously as required, the hairs all develop when the leaf first makes its appearance. Whatever the causes which lead to hair formation they are evidently operative only at the moment when the leaf originates, unless it be that none of the rest of the epidermal cells possess the capacity to produce hairs, or that at a later stage hair formation becomes physiologically impossible.

I. *Hoary.* (Pl. VII, figs. 1—4.)

1. Fully hoary. *M. incana* type, as scarcely needs to be recalled, has all green parts above the cotyledonary level[1], i.e. stem, leaf, axis of the inflorescence, pedicel, calyx and ovary thickly covered with a tomentum which gives the plant a grey appearance (Pl. VII, fig. 1, left-hand pod). As the pod elongates after fertilisation the stigma lobes enlarge to form two pincer-like knobs or horns, which are likewise clothed with hairs though less thickly than the rest of the pod. All four petals in the single and several outer ones in the double[2] sometimes show a sprinkling of hairs in patches here and there on the outer surface (Pl. VII, figs. 2, 3, 4), but quite often they are completely glabrous. Hair

[1] The cotyledons and hypocotyl of every grade including *incana* are absolutely glabrous.

[2] In the earlier account (*Journal of Genetics*, Vol. v. No. 3, p. 146) it is stated that these corolline hairs are confined to the doubles, none having at that time been seen on the singles. It now appears that they are about as frequent and erratic in their distribution in the one case as in the other, and we cannot therefore base any argument as to the more primitive nature of the double on this ground.

development is uniform for all members of a category (stem, leaves, etc.) and is coincident with the coming into being of each member, so that the characteristic full degree of hoariness is to be seen from the moment that the plumule appears between the cotyledons. This is in marked contrast to the progressive development of hairs seen in the following class.

2. Partially hoary. Here the characteristic leaf-surface appearance is only assumed gradually as we pass from the first to the succeeding leaves, and this delayed effect becomes more pronounced as we descend the scale.

(*a*) The half-hoary variety *semi-incana* is chiefly distinguished by having the stem almost devoid of hairs for the greater part of its length. The pedicels, glabrous like the stem on the adaxial, may become somewhat hairy on the abaxial side. The upper surface of the leaves is distinctly less hairy than in the type, so much so as to appear greenish and almost glabrous when seen in contrast with the under surface, which, as well as sepals and ovary, approaches very nearly to that of *incana*. This condition in the case of the leaves is attained by degrees. The first one or two formed by the developing plumule are glabrous except for a few hairs on the apical margin. In each succeeding leaf the hairs become more numerous, extending in those next formed along the whole margin: then, as others follow, appearing on the under surface, thinly at first, more abundantly as we proceed up the stem: finally, as the rosette begins to take shape, becoming numerous on the upper surface also. The stage at which there is a well-developed rosette but as yet no flowering axis is the best for identification. During the flowering period the much-branched stem lessens the general mass effect of the leaves, and later, when axillary shoots begin to develop, their earlier leaves often show a return to the glabrous condition, to be followed only in the ensuing season by others of the characteristic appearance of *semi-incana*. It is as though the forces (factors) governing hair-production either gathered intensity as development proceeded, or as if some inhibitory condition were more pronounced at the beginning of any growth period. (Pl. VII, fig. 1 (middle pod).)

(*b*) The mid-hoary grade, the next in the series and an entirely new form, shows a similar progressive development of hairs, but they are both less abundant and later in making their appearance, and the stationary point is reached at a lower stage. The stem is glabrous, showing few if any scattered hairs below the region of the inflorescence.

Hairs are also as a rule absent from the first two or three leaves. In those formed next the hairs follow the same order of distribution as in *semi-incana*, but there is no ultimate general overspreading of the upper surface. Some hairs may arise, mostly on the midrib and lateral veins, but the green appearance of this side stands in sharp contrast with the grey look of the under surface. Pods and sepals are both hoary, seeming slightly but not markedly less so than in *semi-incana*.

(*c*) The quarter-hoary, a grade lower still in the scale, has been already described[1]. The stem is glabrous and the range of variation in the leaves is again shortened, the period preceding hair development being longer and the maximum level attained lower. The plant, indeed, shows no hairs until the leaf rosette is well developed. A few then appear on the leaf-base margin. In the succeeding leaves which are now attaining the maximum size they begin to extend along the whole length to the apex. Or sometimes the apex may show a terminal hair over the hydathode before this stage has been reached. Both surfaces appear green, the upper being generally quite glabrous, the lower with, at most, a thin sprinkling of hairs, chiefly on the prominent veins. Sepals and pods are less hoary than in the preceding grade, the diminution in the degree of hairiness being most marked in the sepals.

II. *Glabrous.* (Text-figures 1—3, Pls. VII, figs. 1, 5, 6, VIII, figs. 7—9.)

1. Sub-glabrous Group. In this group we may include four grades, all of which, though presenting a glabrous appearance, are not absolutely destitute of hairs throughout. Localisation of the hairs in different regions of the plant in two of these grades as compared with the other two makes impracticable the arrangement of all four forms in one linear series. They fall more naturally into position if we regard the downward trend as diverging here along two descending lines, and the four grades as showing a first and then a further stage in hair reduction as we pursue either direction. In the higher grade in the one case the hairs are detected without great difficulty, but in the other three they are so few as only to be found, as a rule, after tedious search. For this reason, though necessarily classified under the sub-glabrous category, it was found in practice more convenient when dealing with mixed families to include the latter grades in the totals for the wholly glabrous class and to calculate the expectation on this basis. The characteristic features of each grade are as follows:

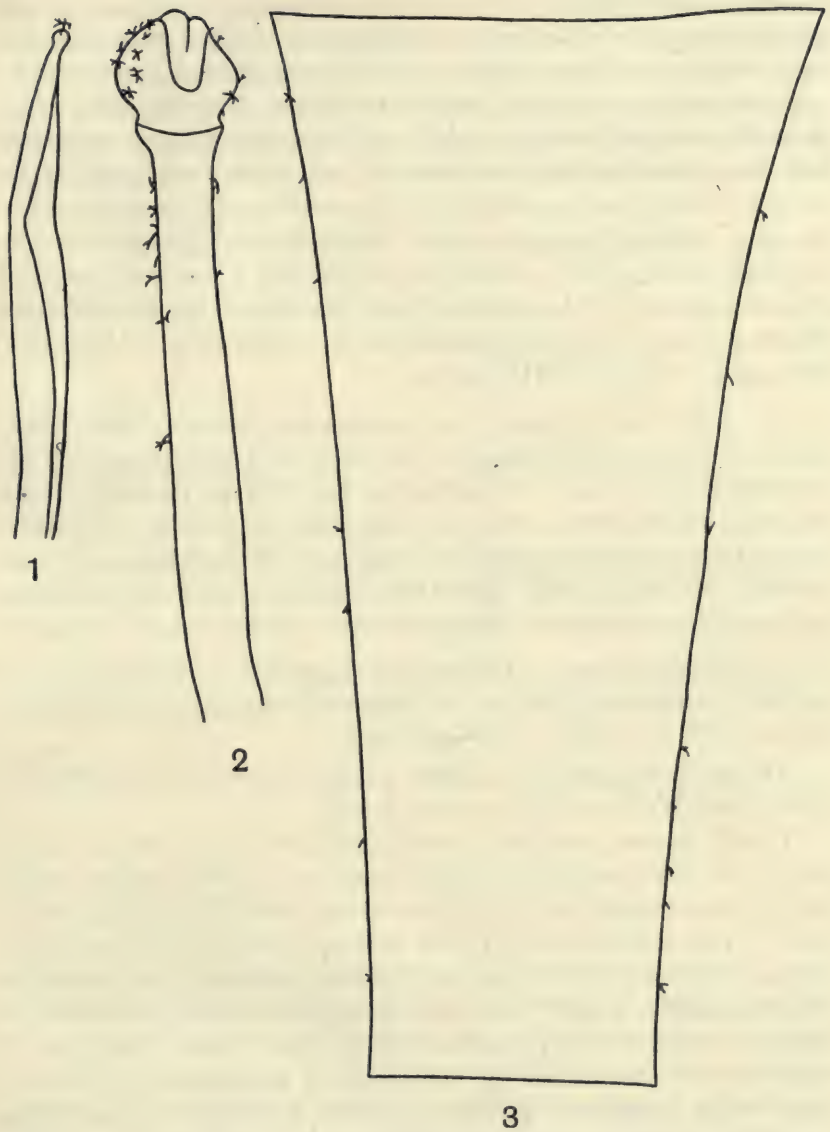
[1] *Journal of Genetics, loc. cit.* p. 152.

(*a*) Here the hairs are restricted to two regions. They are found scattered along the basal margins of some of the larger leaves (Text-figure 3) and distributed very thinly and haphazard over the surface of a few pods, generally one or two of the lowest on some of the earlier flowering axes (Pl. VII, fig. 5). Thus we get here a very considerable drop in the scale. For in the partially hoary grades *every* pod on the individual produces hairs in such abundance as to give a hoary effect, whereas in the present case all but a few are absolutely glabrous, and in these few the hairs are too scanty to produce any mass effect on the eye, and might indeed be counted without much difficulty. Hence the general impression is one of glabrousness throughout.

(*b*) In this grade the plant is all but hairless; only a few solitary hairs are formed on the edges, in the basal region, of a few of the largest leaves. We have the last stage traceable in this direction before final disappearance of the hairy character.

(*c*) This is a particularly interesting form. At the outset of the whole investigation it was noticed that in certain individuals included in the glabrous category either a solitary hair or a small tuft was developed over the region of the watergland at the apex of the leaf[1] (Text-figure 1). Where a lateral vein ends in a hydathode (see p. 155) it also bears a trichome. The occurrence of these hairs appeared at the time to be quite uncertain and without significance, all that could be affirmed was that they occurred in some individuals and not in others, and in the former case on some leaves but not all. We can now give a more precise account of the distribution of these hydathode trichomes and of their value as a diagnostic character. In the various hoary grades where the leaf margins are hairy throughout their length these hairs over the hydathode merge with their neighbours and are not specially noticeable, but in the sub-glabrous individual with the whole leaf otherwise hairless they stand out conspicuously (Pl. VIII, figs. 7 and 8). In plants of this grade the first leaves which succeed the cotyledons as a rule show no hair. Then follow a succession in which these hydathode hairs are a characteristic feature—though occasionally a leaf may be missed, to be followed in their turn as the plant reaches full vigour by many in which they are wanting. Later the hairs again make their appearance on the young leaves of the axillary shoots. The stem, glabrous below, may in well-grown individuals become slightly hairy in the upper internodes (Pl. VII, fig. 6), though in such case still remaining bare as

[1] *Report to the Evolution Committee, Report I*, footnote to p. 33.



Text-figures 1—3 (diagrammatic). 1. Very young leaf seen in profile showing the terminal hydathode and trichome characteristic of the sub-glabrous form containing *CRH*. 2. Typical young siliqua from the same form with the trichomes distributed irregularly along its length and over the stigmatic knobs. 3. Base of a large mature leaf of the sub-glabrous heterozygote $\frac{CRH_1K}{CRh_1k}$ with a few small trichomes scattered along the margin.

a rule beneath each leaf insertion; in less vigorous individuals, e.g. pot-grown plants, it is often hairless throughout. Of the pods some few, mostly the lower ones on the earlier flowering axes, may develop a sprinkling of hairs along the lines of the sutures (Text-figure 2). Such pods when seen in valve view show the hairs projecting on either side, but when turned so that one suture is seen in face view they appear hairless. The enormous majority are however completely glabrous up to the ridge defining the stigma lobes. On the stigma lobes themselves, on the other hand, in all or almost all of the pods a few hairs are to be seen standing out in sharp contrast with the smooth outline of the pod, affording a useful means of identification at a stage when the leaves may fail to give a clue (Pl. VIII, fig. 9).

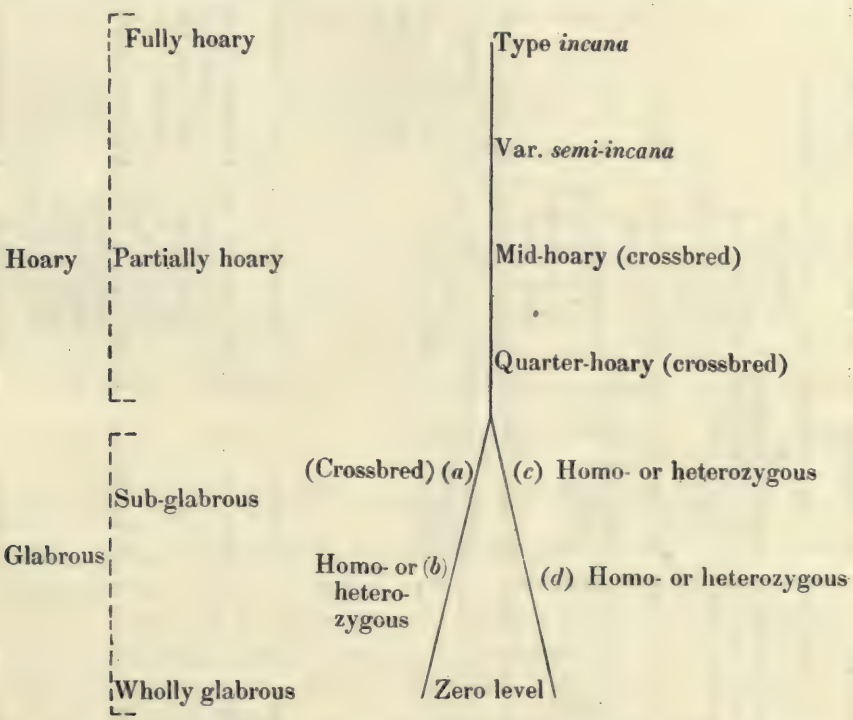
(*d*) This form represents in this direction, like its corresponding grade (*b*) on the other line, the final stage before the complete disappearance of the hairs. On stem and pod they are probably entirely wanting; and whereas on the preceding grade they are *characteristic* of stigma lobe and leaf tip at certain stages, here they are but rarely found in either position, so rarely indeed that discrimination in mixed families between this grade and the wholly glabrous condition was not attempted.

2. Wholly glabrous. The individuals included under this category appear to be entirely destitute of hairs and occupy the zero position in the scale (Pl. VII, fig. 1, right-hand pod).

The serial relation of the whole group of forms here described is made clear in the scheme shown on p. 161.

It will be seen from the descriptions given above that all grades except the two extremes exhibit a range as regards hair production having some relation certainly to the developmental cycle, and possibly also to external conditions. This is most apparent in those forms where the hairy character is on the verge of disappearance. For example in the sub-glabrous grade (*c*) we can clearly see a regular succession of stages as development proceeds, but the even course of this general sequence may be broken by an occasional irregularity, e.g. when a leaf lacking a terminal hair is interposed in a trichome-bearing series: or again—a case perhaps more distinctly traceable to environmental effect—when the later stem internodes of a pot-grown individual remain hairless. Hence for a proper classification of the individual a mere catalogue of the morphological characters without regard to age does not suffice. We must also consider the organism from the physiological and the developmental standpoint. Hardly any one organ, whether leaf,

stem, or pod, regarded as an isolated structure apart from its position in the life-cycle, can be relied upon to reveal the constitution of the intermediate individual. This may be equally true for the whole plant unless it is taken at some critical stage. The regular sequence in development and the irregular effects of what seem to be fluctuations in physiological equilibrium (due to whatever cause) must be taken into



account. Except in the extreme grades at either end of the scale it is the *range* which defines the class, and with an overlapping range throughout the series repeated observations are required as development proceeds. In the scheme given on p. 162 the range of variation for each plant member in each grade is set out so that the combination serving to distinguish each form is appreciable at a glance. For convenience of reference the factorial constitution established as the outcome of the crossbreeding work is also included.

Scheme showing range of variation and factorial constitution of each grade.

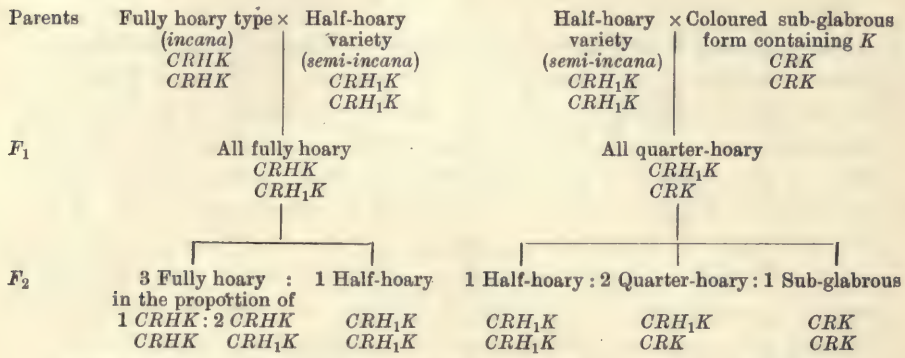
Grade	Factorial constitution*	Characteristics: Stem	Leaf: Under surface	Leaf: Upper surface	Leaf: Margin	Leaf: Hydathode	Siliqua: Pod	Siliqua: Stigma lobes
Type *incana*. Fully hoary	*CRHK* [*CRHK*]	Very hoary throughout	All very hoary ...	All very hoary ...	All very hairy ...	With hairs ...	All very hairy	With numerous hairs
v. *semi-incana*. Half-hoary	CRH_1K [*CR*] H_1K	Glabrous for the most part	No hairs (first leaf) to condition only slightly less hairy than in *incana* (later leaves)	No hairs (first leaf) to condition distinctly less hairy than in *incana* (later leaves)	Few apical hairs (first leaf) to condition very similar to that of *incana* (later leaves)	With hairs ...	All slightly less hairy than in *incana*	With numerous hairs
Mid-hoary. Crossbred	CRH_1K [*CR*] H_1k	Glabrous for the most part	No hairs (first leaf) to condition only slightly less hairy than in *semi-incana* (later leaves)	No hairs (first leaf) to nearly but not quite glabrous (later leaves)	No hairs generally (first leaf) to condition slightly less hairy than in *semi-incana* (later leaves)	With hairs ...	All slightly less hairy than in *semi-incana*	With numerous hairs
Quarter-hoary. Crossbred	CRH_1K [*CR*] h_1K	Glabrous throughout	No hairs (first leaves) to few scattered (later leaves)	No hairs (first leaf) to very few scattered (later leaves)	No hairs (first leaf) to thin sprinkling (later leaves)	No hairs in early leaves Hairs present in later leaves	All moderately hairy throughout	With few hairs
Sub-glabrous. Grade (*c*)	*CRHk* [*CRH*] *k*	Glabrous for the most part, upper internodes in vigorous plants somewhat hairy	Glabrous	Glabrous	Without hairs ...	With hairs in early leaves. Hairs wanting in later leaves	Most glabrous. Few with some hairs on the sutural lines	All or almost all with few hairs
Sub-glabrous. Grade (*d*)	CRH_1k [CRH_1] *k*	Glabrous throughout	Glabrous	Glabrous	Without hairs ...	Rarely with a hair	All glabrous	Rarely with 1–2 hairs
Sub-glabrous. Grade (*a*)	CRH_1K [*CR*] h_1k	Glabrous throughout	Glabrous	Glabrous	No hairs on the enormous majority of leaves. A few with some scattered hairs	All without hairs or possibly an occasional hair may occur	Almost all glabrous. Few with a very thin sprinkling of hairs	Occasionally with hairs on those pods which have hairs
Sub-glabrous. Grade (*b*)	*K* alone or with any combination except *CRH* or CRH_1	Glabrous throughout	Glabrous	Glabrous	No hairs on the enormous majority of leaves. A few with 1–2 solitary hairs	All without hairs	All glabrous	Without hairs
v. *glabra* ...	Any of the remaining factorial combinations	Glabrous throughout	Glabrous	Glabrous	Without hairs ...	All without hairs	All glabrous	Without hairs

* The essential factors are those outside the brackets. Those which, if present in addition, produce no effect are enclosed in brackets. The factor *J* of the earlier account is here represented by H_1.

EVIDENCE FROM FURTHER CROSSBREEDING.

As has been stated above it was already established that full hoariness is dominant to half-hoariness, but the gametic relations of *H* and *J*, the two differentiating factors, were not fully disclosed as no large F_2 generation from a cross between these two forms had then been raised. We now have records of such F_2 families and they show us at once that in respect of *H* and *J* we have to deal with an allelomorphic pair. For among a total of 319 plants composing 7 F_2 families from matings of *semi-incana* and *incana* 246 were fully and 73 half-hoary. (See Experiment 1 in concluding Table.) From the fact that the F_2 generation contains only fully hoary and half-hoary individuals and that these two forms occur in the proportion of about 3 : 1, it is evident that *H* and *J* behave as allelomorphs, and that every gamete of the F_1 individual contains either one or other factor and that none contain both. We have in fact the simple ratio that we expect when the parents differ in respect of *one* factor only. Were *H* and *J* related as distinct factors in the sense in which *H* and *K* are distinct we should expect the F_2 generation to contain a proportion of quarter-hoary $\binom{CRJK}{CRK}$ and glabrous $\binom{CRK}{CRK}$ individuals in addition to the two parental types. Their absence is proof of the alternative distribution of *H* and *J* among the gametes. Behaving as unequally active forms of the same factor such a pair may be likened to the allotropic forms of the chemist. Results in other matings confirm this conclusion. Where a coloured glabrous strain containing *H* is bred with *semi-incana* $(CRH \times CRJK)$ F_1, as before, is fully hoary. In F_2, in accordance with expectation, we find fully hoary, glabrous, and partially hoary plants, but among the latter there are no quarter-hoary, thus proving that here also every gamete must be carrying one or other of the two factors *H* and *J*; otherwise gametic unions in the form $CRJK \times CRK$ would certainly occur and give rise to the quarter-hoary grade. In order that this relation may be plain to the eye an emendation of the original factorial formula is desirable. By employing the symbol H_1 in place of *J* we can display the allelomorphic character of the relation between *H* and the factor which replaces it in the several partially hoary grades and this substitution will henceforward be adopted, and has already been introduced in anticipation in the scheme on p. 162. We shall therefore graphically

represent the factorial relations now shown to be involved in these two unions as set out below:



The allelomorphic nature of the factor H_1 having thus been demonstrated from qualitative results in the cases given above it remains to show in detail that the quantitative composition of the F_2 generation from various unions is closely in accord with expectation based on this relation. Revising our earlier prediction[1] (calculated on a scheme of *distinct* as opposed to *allelomorphic* factors) we shall now restate and amplify this expectation (see p. 165).

In order to obtain proof of the correctness of the expectation there given for the various possible combinations of C, R, H, and K with H_1 it was necessary in the first place to produce individuals which should contain H_1 without either H or K. To this end a glabrous F_2 plant from the mating $CRH \times CRK$ (forms obtainable commercially) found to lack both H and K was bred with *semi-incana*. The resulting cross-bred was then bred back with the glabrous strain CRH, from which mating mixed families were obtained containing about equal numbers of fully hoary and glabrous individuals. As the gametic unions $CRH_1K \times CRH$ and $CRK \times CRH$ would give fully hoary it followed that the approximately equal number of glabrous plants must consist of the two classes $\frac{CRH_1}{CRH}$ and $\frac{CR}{CRH}$. A further back cross between these smooth plants and *semi-incana* yielded again mixed families, some consisting of fully hoary and partially hoary, others of fully hoary and glabrous individuals. Clearly these partially hoary plants which were of a new type must result from the gametic union $CRH_1 \times CRH_1K$. In this way the mid-hoary form was first synthesised and identified, and a pure CRH_1 strain eventually raised. The graphic representation shown

[1] *Journal of Genetics, loc. cit.* p. 157.

Expectation in the case of matings of semi-incana *with* incana *and with the various glabrous and sub-glabrous forms.*

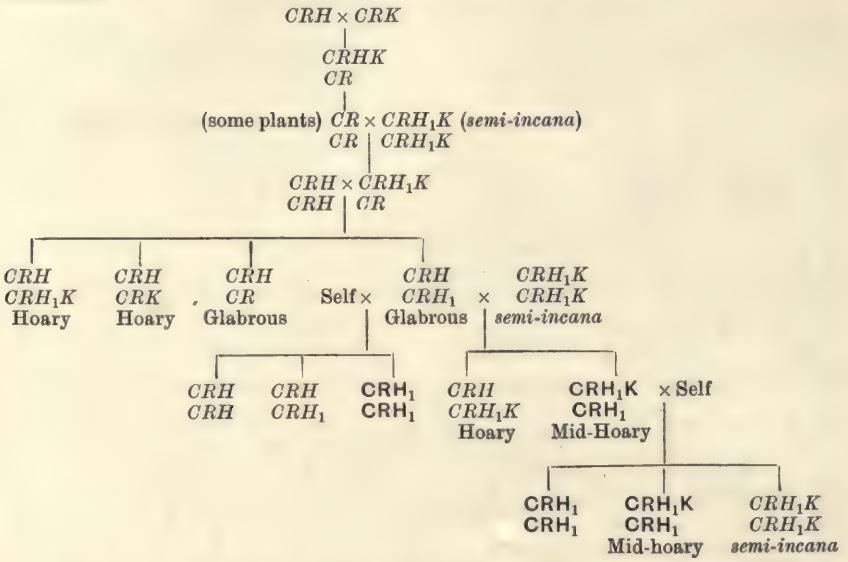
No.	Mating	Expectation in F_1	Expectation in F_2 from self-fertilisation: Hoary	Half-Hoary	Mid-Hoary	Quarter-Hoary	Sub-Glabrous *CRH*	Other Sub-Glabrous and Glabrous
(*a*)	Half-Hoary × Hoary							
1.	**CRH*$_1$*K* × *CRHK*	All Hoary	3	1	—	—	—	—
(*b*)	Half-Hoary × Sub-Glabrous or Glabrous							
2.	**CRH*$_1$*K* × *CRH*	All Hoary	9	1	2	—	3	1
3.	*CRH*$_1$*K* × *CRK*	All Quarter-Hoary	—	1	—	2	—	1
4.	**CRH*$_1$*K* × *CR*	All Sub-Glabrous	—	1	2	2	—	11
5.	**CRH*$_1$*K* × *CHK* or *RHK*	All Hoary	9	3	—	—	—	4
6.	*CRH*$_1$*K* × *CH* or *RH*	All Hoary	27	3	6	—	9	19
7.	*†*CRH*$_1$*K* × *CK* or *RK*	All Quarter-Hoary	—	3	—	6	—	7
8.	*CRH*$_1$*K* × *HK*	All Hoary	27	9	—	—	—	28
9.	*CRH*$_1$*K* × *C* or *R*	All Sub-Glabrous	—	3	6	6	—	49
10.	*CRH*$_1$*K* × *H*	All Hoary	81	9	18	—	27	121
11.	*CRH*$_1$*K* × *K*	All Quarter-Hoary	—	9	—	18	—	37
12.	*CRH*$_1$*K* × —	All Sub-Glabrous	—	9	18	18	—	211
13.	*CRH*$_1$*K* × *CRH*$_1$	All Mid-Hoary	—	1	2	—	—	1
14.	*CRH*$_1$*K* × *CH*$_1$*K* or *RH*$_1$*K*	All Half-Hoary	—	3	—	—	—	1
15.	*CRH*$_1$*K* × *CH*$_1$ or *RH*$_1$	All Mid-Hoary	—	3	6	—	—	7
16.	*CRH*$_1$*K* × *H*$_1$*K*	All Half-Hoary	—	9	—	—	—	7
17.	*CRH*$_1$*K* × *H*$_1$	All Mid-Hoary	—	9	18	—	—	57
(*c*)	(Half-Hoary × Hoary) × Half-Hoary		from a back cross‡					
18.	(*CRH*$_1$*K* × *CRHK*) × *CRH*$_1$*K*	All Hoary	1	1	—	—	—	—
(*d*)	(Half-Hoary × Sub-Glabrous or Glabrous) × Half-Hoary							
19.	(*CRH*$_1$*K* × *CRH*) × *CRH*$_1$*K*	All Hoary	2	1	1	—	—	—
20.	(*CRH*$_1$*K* × *RH*$_1$*K*) × *CRH*$_1$*K*	All Half-Hoary	—	all	—	—	—	—
21.	(*CRH*$_1$*K* × *CR*) × *CRH*$_1$*K*	All Sub-Glabrous	—	1	1	1	—	1

* Indicates those matings in regard to which a restatement of the expectation is necessitated for the reason given above.

† By a printer's error this (the last) mating in the earlier prediction is given as $\frac{1}{2}H \times CR$ instead of $\frac{1}{2}H \times CK$.

‡ As the results have been shown above for every factorial combination in the case of self-fertilisation, the calculation for a back cross is easily made. It is therefore unnecessary to set out the ratios in every case and four instances only are here given in illustration of this type of breeding.

below will enable the successive steps in this operation to be readily followed.



With the new forms thus obtained it became possible to effect any desired factorial combination, and from appearance of the resulting individuals (see the foregoing description and summarised statement set out on p. 162) to group these combinations under the several grades which have now been recognised. In the concluding Table the results of various matings in which the factor H_1 is involved are analysed in detail. In every case it will be seen that there is close agreement between the calculated and the observed results, and the whole body of evidence furnishes sufficient proof of the view here taken of the inter-factorial relations. Numerous confirmatory tests were carried out with "extracted" individuals but as they add nothing to the knowledge already gained it is unnecessary to include them here. It remains briefly to summarise the conclusions drawn from these later experiments and to consider their significance from a physiological aspect.

Summary of Later Results.

1. The type (*incana*) and its wholly glabrous variety are not isolated terms, but the end terms of a series.

2. In both of them the characteristic condition in regard to surface character is exhibited at once in the seedling stage and continues at the same level throughout the life of the plant.

3. Between these two extremes there occurs a series of forms conveniently divided into two groups, the partially hoary and the sub-glabrous.

4. The grades in these two groups, whether homozygotes or heterozygotes, differ from the extreme forms (type and var. *glabra*) in that they exhibit a *progressive* assumption of the characteristic appearance in regard to hairiness.

5. The successive stages overlap to a greater or lesser extent in the several grades; hence periodic observation of the individual as development proceeds may be necessary in order to determine the grade by inspection.

6. In the partially hoary grades the diminished degree of hairiness is due to the presence, in place of the factor H found in the fully hoary type, of an allelomorphic and less potent factor H_1 ($= J$ of the earlier account).

7. The rare and hitherto unnamed variety here designated *semi-incana* is, as its name implies, a partially hoary form, and stands next in the series to the type.

8. The other partially hoary forms, the mid- and quarter-hoary grades, are both heterozygotes, the former as regards one (H_1) of the two contained factors causing hoariness, the latter in regard to the other factor (K).

9. The colour couple CR acts as a limiting factor to the H_1K couple as well as to the HK couple. That is to say the partially hoary condition is only manifested when the whole CRH_1K group is present. (In white-flowered varieties of *incana* and *semi-incana* the colour couple, though ineffective, is nevertheless present, but for some reason not yet clear the production of anthocyanin in the petals is wholly or almost wholly inhibited.)

10. The sub-glabrous grades, though giving the general impression of glabrousness, are not completely destitute of hairs.

11. These grades fall into two groups, showing a different regional distribution of such hairs as occur. Both groups include two grades, the higher showing a slight but still definite degree of hairiness in localised regions either at a certain stage or throughout, the lower, an effect much slighter still, only reaching expression point at certain moments even in the regions in question. In the latter case we have the last stages before total disappearance of the hairs, the upper range limit now rising just above, now falling to vanishing point.

12. Of the 4 sub-glabrous grades one [(a)], like the mid- and

quarter-hoary grades, is a heterozygote containing, like all the partially hoary grades, the 4-factor group CRH_1K, but having only a single dose of both H_1 and K. The slight degree of hairiness manifested is conditioned, as in the partially hoary grades containing a double dose of one or both of these determiners, by the presence of the colour couple. (For these reasons a certain advantage would no doubt have been gained by drawing the arbitrary line between the partially hoary and sub-glabrous categories so as to include this case in the former instead of the latter class, only at the cost however of some absurdity in practice in applying this terminology to a plant so nearly glabrous.)

13. The lower grade in the same linear series [(*b*)] shows the slight effect, only barely perceptible (1—2 solitary hairs on the basal margins of a few leaves), produced by K *either with or without the colour couple*, and independently of H and H_1.

14. The two grades [(*c*) and (*d*)] comprised in the other sub-glabrous group exhibit a distribution of hairs characteristic of H and H_1 *only when present in conjunction with* CR. In this respect therefore both allelomorphs differ from the complementary factor K.

15. All individuals not included in the preceding categories, that is to say all whose factorial constitutions lack K, and also the combination of H or H_1 with CR, appear to be entirely destitute of hairs. They stand at the zero end of the scale and together form the wholly glabrous group.

16. It follows from (13) and (14) that hair production can occur to some slight degree when only one of the two complementary factors for hoariness [H (or H_1) and K] is present.

17. The broad effects resulting from the interaction of the complementary factors for surface character [H (or H_1) and K] with the colour couple (CR) can be stated in general terms thus:

Factorial Combinations	Effect as regards Hair Production
(1) H (or H_1) without CR	Nil
(2) K with or without CR	Minimal, localised in a particular region
(3) H_1 with CR	Minimal, localised in different regions
(4) H with CR	Relatively slight but considerably more extensive than in (2) and (3), localised in several distinct regions
(5) One dose of H_1K with CR	Somewhat similar in magnitude to (4) but localised in different regions
(6) As (5) but with the addition of a second dose of K or H_1 or both	Successively increasing, but still unequally distributed in different regions
(7) HK in any dose with CR	Maximal, overspreading all regions to the fullest extent

DISCUSSION OF THE SIGNIFICANCE OF THE RESULTS.

Apart from the addition to our knowledge of the factorial relations involved which is gained from the observations here recorded, we are able to look upon these results as marking a distinct advance in our view, if not as to the nature of the factors themselves, certainly in regard to the way in which we may suppose them to act. The question whether it may become possible in the future to identify some material component which we can point to as representing this or that factor must be left to the chemist to determine. At the moment the most promising line of investigation seems to me to call for the aid of the physiologist, and until we have assistance in this field I doubt if in the present case any further considerable progress can be made in the direction of distinguishing genetic behaviour from fluctuating variability independent of factorial constitution. It may, however, serve to clear the ground if we attempt to discover what inferences seem to be warranted on this point from the facts already before us.

In the first place whatever the factors already identified may stand for in the Stock it is difficult to resist the conviction that they do not produce their effects, *solely* by causing the production of some particular substance which in their absence is not formed. Of particular interest in this connection is a remarkable plant which appeared unexpectedly in the course of the present work. The individual in question—the only specimen obtained of its kind—was a beautiful example of a mosaic, being in part fully hoary, in part glabrous. The seedling, an F_2 plant from the mating $RH_1K \times CRH$, appeared to be a normal fully hoary individual. Later however, after some 18 or 20 leaves had been formed, two green lines indicating the absence of hairs were observed to extend up the stem, and after widening and converging, finally to coalesce. From this point onwards the mosaic character of the individual was strikingly evident. Leaves arising from this smooth sector all showed glabrous areas of larger or smaller size. In some one side of the midrib would on both surfaces be hoary, the other glabrous. In others sharply defined densely hoary grey patches would be scattered like islands over the surface of the smooth green lamina, sometimes on one surface only, sometimes on both, the areas being sometimes unrelated, sometimes superposed (see Pl. VIII, figs. 10 and 11). A noticeable feature of all such mosaic leaves was the distortion or asymmetry of contour. Where the midrib formed the demarcation line the one side would be well developed with the outline showing an even curved sweep, the other would

be very much narrower with a straight or even concave edge as though growth had been arrested. Where patches of hairs were so distributed as to involve the leaf margin the limit of these areas would be defined by a break in the even contour of the leaf outline, indicating a want of conformity in growth. It therefore seems clear that some difference more profound than the mere localisation of some substance directly causing hair production and one having a marked effect upon growth is here in question.

Another aspect of the question is presented by the following class of facts. We have seen that as regards the character hairiness in the Stock a distinct genotypic maximum can be observed characteristic of the different factorial combinations, but that this maximum is not attained under all circumstances. The nature of the fluctuations suggests that where the genotypic effect is normally slight some change in physiological equilibrium precluding genotypic expression may be brought about by causes unconnected with genotypic constitution. Such equilibrium we imagine to be not a fixed and unvarying condition persisting for any considerable period, but a perpetual balancing of various interdependent and compensating activities resulting in constant, if slight, oscillations about a physiological mean. If a character, regarded as arising in this way, is strictly inherited such oscillations, due to inherent physiological instability, are presumably too slight to affect expression of this genetic character. But we can suppose that these sub-minimal oscillations may become increased by environmental inequalities, and even reach a point which renders somatic expression a physiological impossibility. Or, it may be that the largest deviation so caused in the direction that would lead to physiological inhibition still does not overstep the physiological limit for somatic expression, in which case the genetic forces (factors), with this margin to spare, have still full play and hence produce their maximum effect. The manner in which the hairs are distributed in certain grades points to the conclusion that some connection, however indirect, exists between the hydrostatic conditions set up within the plant, possibly at some critical stage, and the production of hairs. This is most apparent in those forms in which hair formation is so much diminished as to approach vanishing point, as for example in the grade where the leaf shows no hairs except over the hydathodes. If we accept this view it is easy to see how such irregularities as the occasional absence of the terminal hair from a leaf in a trichome-bearing series may be due to some such external cause, e.g. the dryness of the soil acting either directly or indirectly through its effect on a

number of co-ordinated physiological processes. Again such a cause might well account for the absence of hairs from the higher stem internodes frequently observed in pot-grown individuals of the same grade. Although under these conditions the plant often shows a marked modification in habit, producing a much-branched fibrous root-system in place of the few strongly developed roots having scarcely any fibrils usually found in plants grown in the ground, it may well be that perfect compensation in regard to water content is not attained. We can also understand why a particular character phase should recur at a stage in development when we may suppose that a particular growth phase also recurs, as is seen when the hydathode trichomes, occurring on the early leaves of the main axis but not later, reappear on the developing axillary shoots in autumn; or again, when, in those grades in which the bulk of the pods are hairless, the sprinkling of hairs found on the lower fruits on the main axis is sometimes seen again on those occupying a corresponding position on the lateral axes. The idea that the development of hairs is related to varying environmental conditions such as dryness or humidity of the soil and atmosphere is not new. It has been advanced in varying forms by many writers[1]. But the conclusions drawn by different observers from observations of different plants are by no means altogether in accord, and it does not appear that any broad generalisations can be deduced from them apart from the well-known fact that species growing in dry habitats are often characterised by a high degree of hairiness. It is more than doubtful if the explanation of the appearance of hairs which holds good in one species will be found to apply in precisely the same form to another; for the causes may be as diverse in different cases as the causes which lead to doubling in flowers. Most of the observations hitherto recorded deal only with the question of the direct effect of the environment on the production of hairs. The genetic analysis of these forms has yet to be carried out. An interesting case described by Mer[2] which seems to present a certain similarity to that of the sub-glabrous grade of Stock referred to above is that of *Ranunculus aquatilis*. Mer states that he finds that when growing in water this plant has hairs terminating all the leaf segments, even the last, whereas in the terrestrial form the segments are without hairs. The suggestion put forward in explanation

[1] A full account of the literature on this subject up to 1895 is given by Henslow in his "Origin of Plant Structures by Self-Adaptation to the Environment," *International Scientific Series*.

[2] *Bull. de la Soc. Bot. de France*, T. 27, p. 50, 1880.

of the facts seems to me however somewhat strained. Mer's view is that with feeble transpiration the condition of turgescence set up inside the epidermal cells remains constant, growth is uniform, and consequently hairs are not formed: whereas with increased transpiration there may be an accumulation of food materials at certain points which causes the formation of hairs. On this supposition transpiration must be assumed to be more vigorous in the aquatic than in the terrestrial form, a view which seems hardly tenable. In this case, though we have no actual experimental evidence as to the behaviour of the individual when subjected to changed environmental conditions, it may well prove, on investigation, to be comparable with that of *Polygonum amphibium* which has been studied by Massart[1]. According to Massart the hairs produced in this species apart from the flabelliform scales can be classified under three heads: (1) setiform (elongated and tapering to a point), (2) glandular (elongated and terminated by a multicellular gland), (3) scutiform (flat with a base of four cells disposed in a square)[2]. The flabelliform structures occur in every individual. The terrestrial plant also develops hairs of the setiform and scutiform types and under xerophytic conditions it may produce glandular hairs in addition. In the aquatic form only scutiform hairs are found[3]. He has further established by experiment that the individual can be made to assume either the terrestrial or the aquatic habit in direct response to a change of environment. Submerged shoots produced no setiform hairs, but if brought above the level of the water the new growth became hairy. And conversely, subaerial shoots were found always to have setiform hairs, but if submerged they ceased to form hairs on the part developed under water. It is evident that conditions which result in the formation of *scutiform* hairs are set up in the plant under *any* of the environmental conditions described above. Consequently we regard this type of hair as being strictly inherited. In the case of the *setiform* hair the requisite conditions are only established in an aerial medium, hence inheritance is shown only within this *limited range* of environment.

[1] *Bull. Jard. Bot. Bruxelles*, Vol. I. 2, p. 73, 1902.

[2] Massart's figures and description appear to me not to exclude the possibility that the glandular hair is an elaboration of the setiform type and the scutiform hair an arrested form of the flabelliform scale. But whether these epidermal structures are classified under two categories or four is unimportant for the present purpose. The important point is that the species is endowed with more than one kind of hereditary capacity in regard to hair formation.

[3] The further and more profound differences characterising these forms are shown in Massart's figure, p. 75.

Massart considers that in these circumstances it is difficult to hold that this character is inherited at all, and sums up the position with the statement that the more free a species is from the shackles of heredity the greater is its faculty for accommodating itself to the environment. In the light however of what we know from genetic analysis in other cases we may view the above facts from a somewhat different standpoint. We may presume that in *Polygonum* when submerged, and in *Ranunculus* when growing out of water, the hereditary mechanism is unable to bring about the physiological conditions necessary for the development of the hairs in question. The portion of the mechanism immediately concerned in the formation of scutiform hairs in *Polygonum* may be looked upon as in a state of such stable adjustment that its working is unaffected by changes in the external medium; it is always effective. But another piece of the mechanism involved in setiform hair production may be imagined to be so delicately balanced that a change of environment causes a readjustment from a hair-producing to a non-hair-producing state of equilibrium, or *vice versa*. To this extent we may agree that inheritance is not shown. But if the requisite physiological condition for hair development is set up by suitable environmental conditions the hereditary mechanism comes into play, determining the form (and in *Ranunculus* the position also) of the hairs. In the same way we may explain the effects which result in certain plants from injury. Injury in the case of a hairless form may result in the setting up of an altered condition of physiological equilibrium which permits of hair formation, the character of the hairs which result, however, being clearly determined by some hereditary process. It is stated, for example, that galls formed on the glabrous species *Vitis vulpina* and *V. cordifolia* show hairs resembling those forming the tawny tomentum of *V. aestivalis* and *V. Labrusca*; and that hairs produced through insect activity on smooth-leaved Oaks are of the stellate type ordinarily found in hairy-leaved Oaks[1] and other instances could be given. In this connection may also be mentioned the interesting results obtained by A. W. Hill[2] with *Tropaeolum peregrinum* L. (Canary-creeper). Hill found that it was possible to induce this glabrous species to develop hairs by removing the lamina of the leaves, leaving only the mid-rib. In none of these cases, however, do we yet know the exact nature of the change which is induced.

The expenses incurred in connection with this work have been defrayed in part by grants from the Royal Society and from the British Association for the Advancement of Science.

[1] See Coulter, Barnes, and Cowles, *Textbook of Botany*, Vol. II, p. 575, 1910–11.

[2] *Annals of Botany*, Vol. XXVI. 1, p. 589, 1912.

EXPERIMENTAL DATA.

TABLE I.

Results obtained in the case of matings of semi-incana *with* incana *and with the various glabrous and sub-glabrous forms*.*

Expt.	Mating	F_1	F_2 from self-fertilisation						
		Hoary	Hoary	Half-Hoary	Mid-Hoary	Quarter-Hoary	Sub-Glabrous *CRH*	Other Sub-Glabrous and Glabrous	
(*a*)	Half-Hoary × Hoary								
1.	$CRH_1K \times CRHK$	22	77	25	—	—	—	—	
		—	36	12	—	—	—	—	
		—	17	5	—	—	—	—	
	Reciprocal	42	45	10	—	—	—	—	
		—	45	15	—	—	—	—	
		—	23	5	—	—	—	—	
		—	3	1	—	—	—	—	
	Totals	64	246	73	—	—	—	—	
	Expectation ...	all Hoary	239	80	—	—	—	—	
(*b*)	Half-Hoary × Sub-Glabrous or Glabrous; or other unions yielding equivalent factorial combinations								
2.	†$CRH_1K \times CRH$	22	140	14	30	—	44	17	2‡
		—	51	3	5	—	19	12	—
	Reciprocal	25	139	18	40	—	57	19	5‡
		—	111	17	31	—	33	12	7‡
	Totals	47	441	52	106	—	153	60	14
	Expectation ...	all Hoary	465	52	103	—	155	52	—
3.	$CRK \times CRH_1K$	Quarter-Hoary 29	(See earlier account, *loc. cit.* p. 153, Expts. 9 and 14)						
	Total	29							
	Expectation	all Quarter-Hoary	—	1	—	2	—	1	
4.	$CR \times CRH_1K$	Sub-Glabrous 12	—	3	4	6	—	35	
		4	—	7	12	16	—	68	
		9	—	10	16	22	—	132	
		2	—	15	30	33	—	154	
		4	—	16	38	29	—	162	
		1	—	11	26	29	—	99	
		1	—	5	14	8	—	73	
		1	—	12	26	24	—	140	
		—	—	3	6	5	—	33	
	Totals	34	—	82	172	172	—	896	
	Expectation	all Sub-Glabrous	—	83	165	165	—	909	

* These results are additional to those previously recorded. The recognition of, and classification into, a larger number of categories made the inclusion of the earlier records in the present analysis impracticable, though the size of these families was in any case too small to have any real value (*loc. cit.* p. 153, Experiments 7—11).

† In the earlier account (*loc. cit.* p. 153, Experiment 7) an F_2 individual from a mating of this kind was recorded as quarter-hoary. In reality no doubt the plant will have belonged to the mid-hoary class, a grade which at the time had not yet been recognised. This remark applies equally to the record of quarter-hoary plants in Experiment 8 of the earlier account.

‡ Died young, either *CRH* or CRH_1.

TABLE I (*continued*).

Expt.	Mating	F_1	F_2 from self-fertilisation					
		Hoary	Hoary	Half-Hoary	Mid-Hoary	Quarter-Hoary	Sub-Glabrous *CRH*	Other Sub-Glabrous and Glabrous
5.	**CRH*$_1$*K* × *RHK*	9	24	12	—	—	—	18
		—	75	24	—	—	—	38
		—	45	13	—	—	—	29
	Reciprocal	11	127	41	—	—	—	58
	Totals	20	271	90	—	—	—	143
	Expectation ...	all Hoary	283·5	94·5	—	—	—	126
6.	*CRH*$_1$*K* × *RH*. This precise mating was not carried out, but the same combination of factors was obtained in another way, as shown							
		Hoary						
	RH$_1$*K* × *CRH*	6	70	6	10	—	21	58
	Reciprocal	5	—	—	—	—	—	—
	Totals... ...	11	70	6	10	—	21	58
	Expectation	all Hoary	70	8	15	—	23	49
		Quarter-Hoary						
7.	*CRH*$_1$*K* × *CK*	11	(See earlier account, *loc. cit.* p. 153, Expt. 10)					
	Reciprocal	17	—	—	—	—	—	—
	Totals	28	—	—	—	—	—	—
	Expectation	all Quarter-Hoary	—	3	—	6	—	7
		Sub-Glabrous						
8.	*CRH*$_1$*K* × *R*	15	—	14	29	28	—	219
		—	—	1	4	4	—	20
		—	—	1	1	2	—	22
	Reciprocal	6	—	5	8	12	—	97
		—	—	9	25	20	—	174
		—	—	5	10	4	—	48
	RK × *CRH*$_1$	6	—	11	15	16	—	130
		—	—	6	16	16	—	80
		—	—	5	10	8	—	53
		—	—	4	10	10	—	53
		—	—	6	9	9	—	48
		—	—	4	8	6	—	65
	Totals	27	—	71	145	135	—	1009
	Expectation	all Sub-Glabrous	—	64	127·5	137·5	—	1041
		Sub-Glabrous						
9.	—— × *CRH*$_1$*K*	41	—	4	10	11	—	100
		—	—	5	11	16	—	139
		—	—	12	24	22	—	296
		—	—	10	22	19	—	191
		—	—	4	3	11	—	74
		—	—	1	1	7	—	71
		—	—	3	7	8	—	147
		—	—	2	3	3	—	57
	Totals ...	41	—	41	81	97	—	1075
	Expectation	all Sub-Glabrous	—	45	91	91	—	1067

* In regard to the result recorded for this mating in the earlier account see note to Experiment 2 in this Table.

TABLE I (*continued*).

Expt.	Mating	F_1	F_2 from self-fertilisation					
		Mid-Hoary	Hoary	Half-Hoary	Mid-Hoary	Quarter-Hoary	Sub-Glabrous *CRH*	Other Sub-Glabrous and Glabrous
10.	$CRH_1 \times CRH_1K$	9	—	6	22	—	—	4
		—	—	3	13	—	—	4
		—	—	4	11	—	—	9
		—	—	3	13	—	—	12
		—	—	3	13	—	—	5
		—	—	10	14	—	—	5
		—	—	7	12	—	—	9
		—	—	1	1	—	—	1
		—	—	6	11	—	—	7
	Reciprocal	5	—	4	14	—	—	7
		—	—	10	15	—	—	5
		—	—	6	9	—	—	5
		—	—	1	2	—	—	1
		—	—	3	15	—	—	6
	Totals	14	—	67	165	—	—	80
	Expectation ...	all Mid-Hoary	—	78	156	—	—	78
		Half-Hoary						
11.	$CRH_1K \times RH_1K$	1	—	65	—	—	—	23
	Totals	1	—	65	—	—	—	23
	Expectation ...	all Half-Hoary	—	66	—	—	—	22

		F_2 from a back or other second cross					
(*c*)	(Half-Hoary × Hoary) × Sub-Glabrous						
12.	$(CRH_1K \times CRHK) \times (RH_1K \times RK)$	11	4	—	7	—	—
	Expectation ...	11	5·5	—	5·5	—	—
	$(CRH_1K \times CRHK) \times CRK$	14	—	—	14	—	—
	Expectation ...	14	—	—	14	—	—
(*d*)	(Half-Hoary × Sub-Glabrous *CRH*) × Half-Hoary						
13.	$(CRH_1K \times CRH) \times CRH_1K$	48	19	29	—	—	—
	(Reciprocal) × ,,	10	3	3	—	—	—
	Totals	58	22	32	—	—	—
	Expectation ...	56	28	28	—	—	—
14.	(Half-Hoary × Sub-Glabrous *CRH*) × Sub-Glabrous						
	$(CRH_1K \times CRH) \times K$	27	—	—	4	—	16*
	Expectation ...	24	—	—	12	—	12
15.	(Half-Hoary × Sub-Glabrous) × Half-Hoary						
	$(CRH_1K \times RH_1K) \times CRH_1K$	—	25	—	—	—	—
	Expectation ...	— all Half-Hoary —			—	—	—
16.	Sub-Glabrous × (Sub-Glabrous *CRH* × Half-Hoary)						
	$RK \times (CRH \times CRH_1K)$	35	—	—	16	—	19
		20	—	—	7	—	7
		16	—	—	8	—	7
	Reciprocal	45	—	—	12	—	13
	Totals ...	116	—	—	43	—	46
	Expectation...	103	—	—	51	—	51

* Several of these glabrous plants died as seedlings. Had they lived it is probable that some would have been found to belong to the quarter-hoary class.

TABLE I (*continued*).

Expt.	Mating		F_2 from a back or other second cross					
			Hoary	Half-Hoary	Mid-Hoary	Quarter-Hoary	Sub-Glabrous *CRH*	Other Sub-Glabrous or Glabrous
17.	(Glabrous × Half-Hoary) × Half-Hoary							
	$(CR \times CRH_1K) \times CRH_1K$		—	7	4	4	—	4
			—	10	11	7	—	17*
		Totals ...	—	17	15	11	—	21
		Expectation		16	16	16	—	16
18.	Sub-Glabrous × (Sub-Glabrous *CRH* × Sub-Glabrous)							
	$RK \times (CRH \times RH_1K)$		42	—	—	17	—	13
			34	—	—	12	—	20
		Totals ...	76	—	—	29	—	33
		Expectation	69	—	—	34·5	—	34·5
19.	Sub-Glabrous *CRH* × Mid-Hoary							
	$CRH \times CRH_1KCRH_1$		18	—	—	—	18	—
		Expectation	18	—	—	—	18	—
20.	Mid-Hoary × Sub-Glabrous							
	$CRH_1KCRH_1 \times CRH_1$		—	—	4	—	—	4
	Reciprocal		—	—	20	—	—	25
		Totals ...	—	—	24	—	—	29
		Expectation	—	—	26·5	—	—	26·5
21.	Sub-Glabrous *CRH* (hybrid) × Half-Hoary							
	$CRHCRH_1 \times CRH_1KRH_1K$		11	—	13	—	—	—
			11	—	16	—	—	—
			15	—	16	—	—	—
		Totals ...	37	—	45	—	—	—
		Expectation	41	—	41	—	—	—

* See note on preceding page.

EXPLANATION OF PLATES VII AND VIII.

Fig. 1. Siliqua from a fully hoary (left), a partially hoary (centre) and a glabrous type (right).

Fig. 2. Unopened bud of single flower from a fully hoary plant showing a few scattered hairs on the outer surface of the petals. (Well seen in the region indicated by the arrow.)

Fig. 3. Open single flower of a fully hoary plant seen from the back with hairs scattered here and there on the under surface of the petals. (Seen most clearly on either side of the midrib in the lower right-hand petal—indicated by arrow.)

Fig. 4. Portion of double flower of a fully hoary plant seen from the back. One patch of scattered hairs is to be seen on the median petal (indicated by arrow), near the base, and another towards the free border. Smaller patches occur on several other petals.

Fig. 5. Leaf base and pod from the sub-glabrous grade (*a*). A few solitary hairs occur on the leaf margin and here and there in the middle region of the pod.

Fig. 6. Upper stem internode, slightly hairy, from the sub-glabrous grade (*c*).

Figs. 7, 8. Leaves of the sub-glabrous grade (*c*) hairless except for the hydathode trichomes.

Fig. 9. Characteristic pods from the same with conspicuous hairs on the stigmatic horns and sutures.

Fig. 10. Leaf from a mosaic plant showing the markedly unequal development of the hoary (left) and glabrous (right) halves.

Fig. 11. Two leaves from the same showing small irregular hairy patches. Where such a patch involves the actual margin the limit of the patch is often defined by an irregularity in the contour line.

Fig. 1.

Fig. 5.

Fig. 3.

Fig. 6.

Fig. 2.

Fig. 4.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

VOLUME X OCTOBER, 1920 No. 3

RACIAL STUDIES IN FISHES.

IV. EXPERIMENTAL INVESTIGATIONS WITH *ZOARCES VIVIPARUS* L.

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(With 2 Text-figures.)

I. INTRODUCTION.

IN a previous paper (*Journal of Genetics*, Vol. VII, 1918) the principal results of some statistical investigations with *Zoarces viviparus* were summarized. I there expressed the following view, with regard to the nature of "races" in *Zoarces* and other fishes as characterised by our population-analyses (l.c. p. 117): "A fish 'race' is largely a statistical conception. It implies a mixing of different genotypes, and the average values characterising the 'race' are primarily dependent upon the quantitative proportion between these; only secondarily on the environment." It was further pointed out that we could not expect to arrive at any final decision before the results of those experimental investigations which were carried out along with the statistical investigations with the same species since 1914, were available. This is the case now, at least with regard to some of the experiments which are sufficiently advanced as to be suitable for publication. I propose to give here a summary of some of the most important results. A detailed account will be published later on in the *Comptes rendus des travaux du Laboratoire Carlsberg*, Copenhagen.

Two *Zoarces* populations are to be dealt with here, both from Danish waters, viz. the Ise Fjord and the Roskilde Fjord, north coast of Sealand, the latter being a branch of the former (see Fig., p. 186). Both populations were mentioned in the paper quoted above and graphs representing the number of vertebrae were given (l.c. p. 110, Fig. 4) from the Ise Fjord (Station 31) and further Fig. 6 (to the right) from the Roskilde Fjord (Station 34).

In addition to the ordinary population-analyses *individual offspring analyses* have been carried out each autumn since 1914, the offspring of each gravid female being separately investigated. From each female, 10 embryos (a "decade") were taken at random and their number of vertebrae etc. determined[1].

For the sake of brevity I shall restrict myself to mentioning here a single character, viz. the *number of vertebrae*. This character has proved to be of great importance in distinguishing local races in *Zoarces*, where the number has been found to vary from 99 to 126. A large mass of material of many thousands of specimens was analysed and statistically treated. The countings were made by myself (1914–1917) and by Mr Vilhege, M.Sc. (1917–1919).

II. Comparison of the Different Annual Classes within a Population.

For this purpose the *decade investigations*, consisting in an analysis of ten embryos taken at random from each gravid female, are very well suited. By examining the embryos instead of the adult specimens all doubt is of course excluded that the individuals belong to the same annual class.

Table I (p. 188) shows the results of offspring-analyses (decade investigations) within the population from Station 31 during the years 1914–1919. Altogether 871 decades or 8710 individuals rather evenly distributed between the six years have been examined.

It is seen from this table that the population-averages for the different annual classes agree very well. By means of the present great mass of observations it has thus been possible to point out that there is no difference between the various annual classes with regard to the average number of vertebrae. In other characters such a difference can, however, be pointed out, e.g. for the number of pectoral rays[2].

In Table II (p. 189) the same material is arranged in another way. If the figures are set out as a curve it will be seen that this is very regularly shaped, as might be expected where the material is so large.

Table III (p. 189) also deals with the same material and shows the average values of ten decades, each figure representing a hundred individuals. It will be seen that the highest value is 114·17, the lowest

[1] See Johs. Schmidt: "*Zoarces viviparus* L. and local races of same" (*C. R. Laboratoire Carlsberg*, Vol. XIII, p. 389, Copenhagen, 1917).

[2] See my paper in *Comptes-Rendus du Laboratoire Carlsberg*, Vol. XIV, No. 1, pp. 2–11, 1917.

112·28 which are respectively 0·97 above, and 0·92 below the average of the population which is 113·1963. The values of Table III are especially interesting when compared with the corresponding figures for individuals coming from the same population, but developed under different external conditions (see Section IV, p. 182).

III. Comparison of Mothers and their Offspring. Significance of Internal Factors.

This part of the investigation is directed to the question whether the character examined, viz. the number of vertebrae, is hereditary, i.e. due to internal factors. As the father in each single case is unknown it is only possible to compare the offspring with the mother. As the material is so large we can however assume that the paternal number of vertebrae is on an average equal to the average of the population, i.e. about 113·20. If the number of vertebrae is a hereditary character our investigation should show that mothers with a great number have offspring with a great number. To test this we arrange the material given in Tables I–III so that for each number of vertebrae occurring in mothers we indicate the frequency of it and the average number of vertebrae in the offspring derived from these mothers.

From Table IV, representing a very considerable material, namely no less than 857 mothers and 8570 individuals of their offspring, it clearly appears that the average number of vertebrae in the offspring depends upon the number of vertebrae of the mothers, since the values of the offspring continuously increase as the maternal numbers of vertebrae increase from 107 to 119. Such a conformity could not appear if the number of vertebrae in an individual were alone determined by the environmental conditions during the development.

The investigations with *Zoarces* thus show that there are differences of hereditary nature between the various individuals, and they confirm the results of R. C. Punnett, arrived at by investigation of the viviparous shark *Spinax niger*[1], as well as those obtained from my experiments with *Lebistes* and the common trout (this *Journal*, Vol. VIII, No. 3, 1919 and Vol. IX, No. 1, 1919).

The Table thus shows that the number of vertebrae in *Zoarces* is a hereditary character. We must, however, not expect that the mothers

[1] R. C. Punnett: "Merism and Sex in *Spinax niger*" (*Biometrika*, Vol. III, Part IV, 1904).

with for instance 119 vertebrae have offspring with on an average 119 vertebrae. As will be seen from the table that is not the case, the average being only 116·3. In the first place the paternal number is not 119, but about 113·2 (the average of the population), a circumstance that causes the offspring to "regress" towards the average value of the population. Further "regression" is due to the fact that the maternal numbers of vertebrae, for example, 119, are personal values, while their generative values in all probability will deviate less from the average of the population than do the personal values[1]. Provided the material be sufficiently large, the following relation must presumably hold:

$$\tfrac{1}{2}(\text{Average } ♀♀\ 119 + 113{\cdot}2) = \text{Average Offspring of } ♀♀\ 119,$$

where "Average ♀♀ 119" is the average of the generative values of mothers with 119 vertebrae and "Average Offspring of ♀♀ 119" is the average of the offspring originating from those mothers.

I am not going to discuss the results of Table IV more closely here. As the present large material, however, seems to deserve a more thorough statistical treatment than I am prepared to give it I have asked the statistician Miss Kirstine Smith, D.Sc. to deal with it and I hope the results will before long be ready for publication in the *Comptes Rendus du Laboratoire Carlsberg.*

IV. Comparison of Two Different Populations developed under the same Environment. Significance of Internal Factors, *continued.*

The two populations in question differ essentially in the average number of vertebrae, which for the first of them, the population dealt with above, from the mouth of the fjord (Station 31) is about 113·2, and for the other population, from the innermost part of the fjord (Station 34, see Fig. 1, p. 186) about 108·0. The experiment was carried out thus. In June 1916, i.e. at a time of year when the sexual products of *Zoarces* are not yet ripe, about 300 specimens of each population were caught. They were removed to an embanked area in the neighbourhood of Station 31 and put into big perforated wooden boxes, placed on the

[1] About the concepts "personal" and "generative" value see Johs. Schmidt: "Racial Studies in Fishes," III (this *Journal*, Vol. IX, p. 63, 1919) and "La valeur de l'individu à titre de générateur, appréciée suivant la méthode du Croisement diallèle" (*C. R. Laboratoire Carlsberg*, Vol. XIV, No. 6, Copenhagen, 1919).

I take the occasion to correct a slip in the footnote p. 67 of the first paper. In the third line from the bottom instead of *accuracy* read *probable error.*

bottom near each other, projecting above the water so that feeding could easily be carried out. The pairing and the development of the embryos thus took place in captivity and under the same external conditions for both population-samples. In December 1916, i.e. at a season when *Zoarces* contains large embryos, the boxes were lifted out of the water and their contents preserved. In the population-sample from Station 31 was found 27 females with embryos, and in that from Station 34 a number of 11.

Under natural conditions the average number of vertebrae in the population at Station 31 is about 113·2 (see Table I), while in the population at Station 34 it is about 108·0 (see my first paper in this *Journal*, Vol. VII, 1918, the graphs p. 110 (Station 31) and the graph to the right at the foot of the table inserted between pp. 110 and 111 (Station 34, average 107·98)). The values for the two populations thus differ by about 5 vertebrae, a difference which the annual investigations since 1914 have proved constant. In spite of the small distance of about 50 kilometers between the two stations, their natural conditions differ widely, e.g. at the first of them the salinity of the water is about 21 ‰, and at the other only about 12 ‰. It might therefore be conceivable that the cause of the difference in the number of vertebrae would be found in the different environmental conditions. If this was the case, it was to be expected that the difference would disappear when the two populations were brought under similar external conditions. This was done in our transplantation experiment, and it is the question now, whether the difference of 5 vertebrae, appearing under normal conditions, has disappeared in the offspring, all of which have developed in our experiment under the same external conditions.

The result of the experiment is given in Table V (p. 190). For comparison it may be stated that the average number of vertebrae in offspring developed under natural conditions in 1916 for Station 31 was 113·101 ± 0·447[1] (125 decades), and for Station 34 was 107·768 ± 0·468 (116 offspring-samples each consisting of 5 specimens), showing as usual a difference of about 5 vertebrae.

It is seen from the table that the offspring of the transplanted population sample from Station 31 had on an average 114·819 ± 1·059 vertebrae, while the corresponding value for the sample from Station 34 was 108·283 ± 1·363. In other words the difference between the two popu-

[1] The uncertainty of the average is here and in the following indicated by the fluctuation equal to 5 times the probable error (see my first paper in this *Journal*, Vol. VII, No. 2, p. 118 note, 1918).

lations *has not disappeared* although the development took place under the same circumstances. The result of our transplantation experiment with *Zoarces* thus agrees with the experiment with *Lebistes* previously described (see this *Journal*, Vol. VIII, pp. 150–153, 1919) and confirms the conclusion that the difference, proved to exist between the two populations, is of hereditary (genotypical) nature.

V. Comparison of Two Samples of the same Population developed under different Environmental Conditions. Significance of External Factors.

The transplantation experiments just mentioned were continued in 1917 with material from Station 31. A great number of *Zoarces* individuals were in early summer, and therefore when the sexual organs were still unripe, brought into four large wooden boxes placed in the same embanked area near Station 31 as in 1916.

When the experiment was brought to a conclusion the total number of gravid females was 92. The number of offspring samples examined, derived from transplanted specimens, was, including the 27 decades from the 1916 experiment, altogether 119.

The investigation showed that the average number of vertebrae of the 119 decades was $114{\cdot}535 \pm 0{\cdot}493$[1]. The corresponding figure for the non-transplanted samples of the same population was in 1916 $113{\cdot}101 \pm 0{\cdot}447$ and in 1917 $113{\cdot}251 \pm 0{\cdot}372$. It is therefore evident that the external conditions, altered through the transplantation, have *decidedly raised the average number of vertebrae* in the population.

The same thing becomes apparent when the material is arranged in another way. With the same arrangement of the material from the transplantation experiment as was used in Table III, we arrive at the following figures, each of which represents 100 individuals (10 decades):

114·63	114·08
114·55	114·59
114·65	114·65
114·09	114·46
114·09	114·42
113·88	114·42 (average of 9 decades only).

[1] For the sake of completeness it is stated that the average for the transplanted mothers was $112{\cdot}74 \pm 0{\cdot}65$, and for the males (111 specimens), found in the boxes at the conclusion of the experiment, $112{\cdot}95 \pm 0{\cdot}61$.

These figures have to be compared with the figures of Table III for non-transplanted samples. Such a comparison does not leave any doubt that the number of vertebrae has really been raised by the transplantation: out of the 12 values above 11 are greater than 114, while of the 87 values in Table III only 5 are greater than 114.

It is further shown in Table VI (p. 190) that the average number of vertebrae in offspring of mothers with a given number of vertebrae, e.g. 116, is greater for transplanted than for non-transplanted specimens.

It is therefore proved to a certainty that the transplantation from the natural conditions at Station 31 to the embanked area raises the average number of vertebrae in the population. It is not possible to decide for certain what factor or factors contribute to that effect. But it is a fact that the temperature in the boxes, placed in quite shallow water, about $2\frac{1}{2}$ meters deep, was higher than at Station 31 and on the whole probably higher than for any natural population of *Zoarces* in Danish waters. On the other hand the salinity was lower in the boxes than at Station 31, viz. about 12 $^{o}/_{oo}$ against about 21 $^{o}/_{oo}$ at Station 31. In the embanked area, where the boxes were placed, and which is used as an eel-farm, *Zoarces* is only found occasionally and in extremely small numbers, whereas it abounds outside the dam, at Station 31.

VI. THE *ZOARCES* POPULATIONS OF THE ISE FJORD—ROSKILDE FJORD AREA.

Beside the yearly analyses of the populations from Stations 31 and 34 we in 1919 carried out analyses of a greater number of populations from the same fjord area. As will be seen from Fig. 1 the Ise Fjord opens into the Kattegat (at the north coast of the isle of Sealand), and Roskilde Fjord is a branch of Ise Fjord. In both fjords *Zoarces* occurs in abundance right from the mouth to the base.

Table VII (p. 191) shows the result of our population-analyses which are also represented in Fig. 1, p. 186. It will be seen that the populations living outside the two fjords, in the Kattegat, have the average number of vertebrae which is characteristic of the populations of the open sea in the Baltic area, viz. 117–118. In the mouth of the fjord the number is 114–115. From this the value falls quite evenly in the Ise Fjord to 112·2, which is the lowest value for that fjord. In the Roskilde Fjord it falls suddenly to 111·2 (Station 71) and 110·9 (Station 72), reaching near the base of this fjord, at Station 34, the lowest average value hitherto known for any *Zoarces* population, viz. about 108·0.

This detailed investigation thus quite confirms the results of my previous investigations (see this *Journal*, Vol. VII, 1918, p. 111) and shows strikingly in all details how the average number of vertebrae

Fig. 1. Average number of vertebrae in 11 *Zoarces* populations from the Ise Fjord—Roskilde Fjord area, Sealand, Denmark.

The figures at the black spots indicate station numbers, those within the rectangles the average number of vertebrae of the population concerned.

decreases from the coast and the mouth of the fjord towards the base of the fjord. Here *Zoarces* has a short and plump shape, while the populations living farther out consist of individuals slender and elongated in

shape (see this *Journal*, Vol. VII, 1918, Plate VII). This quite regular gradation in the average number of vertebrae has now been found in all Danish fjords where investigations have been undertaken and it must therefore be considered a definitely established fact.

Fig. 2. Ise Fjord—Roskilde Fjord area, Sealand, Denmark.
Showing the salinity of the water in per mille (‰) at the end of November, 1919.

The chemical and physical conditions of our fjords are not closely investigated. Among the conditions which are best elucidated the salinity of the water exhibits the most characteristic picture. As will be seen from Fig. 2 this factor shows a gradation which accurately

corresponds with the gradation in the average number of vertebrae. To conclude from that fact that this character is directly determined by the salinity or by a factor acting parallel to it—which several investigators have been inclined to do—is however not justifiable as I have already explained in my first paper in this *Journal* (Vol. VII, 1918, pp. 115–117).

VII. Concluding Remarks.

The investigations and experiments with *Zoarces* described above have provided new evidence of the correctness of the view that the race-characters in fishes are of a hereditary nature, i.e. that they are first and foremost determined by internal factors (Sections III and IV). That they are however on the other hand to a certain extent influenced by external factors, is here for the first time experimentally proved for one of our northern species of fish. We have thus seen that the average number of vertebrae which under natural conditions remained very constant in six consecutive annual classes of the same population (Section II) was altered, if not strongly, at any rate perceptibly, by a transplantation of the population (Section V).

In view of the investigations hitherto undertaken the gradation of the average number of vertebrae has to be interpreted not as a direct effect of the external conditions (the salinity or another factor acting parallel to it), but as the result of a selection.

TABLE I.

Station 31, *Ise Fjord, Denmark*, 1914—1919. *Decade Investigations.*

Average number of vertebrae (a) in the six annual classes 1914—1919.

n number of decades, a average number of vertebrae, σ standard deviation for average value of a decade, P.E.A. probable error of average, P.F.A. probable fluctuations of average.

Year	1914	1915	1916	1917	1918	1919	1914—1919
n ...	139	177	125	178	154	98	871
a ...	*113·1799*	*113·2746*	*113·1008*	*113·2506*	*113·2481*	*113·1235*	*113·1963*[1]
σ^2 ...	1·452	1·459	1·480	1·472	1·453	1·265	—
P.E.A.[2]	0·083	0·074	0·089	0·074	0·079	0·086	0·032
P.F.A.[2]	0·415	0·370	0·447	0·372	0·395	0·431	0·160

[1] Average of six averages (1914—1919).

[2] Calculated from exact decade averages.

TABLE II.

Station 31, *Ise Fjord, Denmark*, 1914—1919. *Decade Investigations.*

Number of vertebrae in 857 decades. Same material as in Table I[1].

Number of Vertebrae	Number of Specimens
122	1
121	1
120	6
119	22
118	100
117	269
116	647
115	1109
114	1616
113	1785
112	1397
111	893
110	463
109	172
108	65
107	17
106	6
105	1

[1] 14 decades—offspring of 14 mothers in which the number of vertebrae could not be determined due to accidental damage—were excluded.

TABLE III.

Station 31, *Ise Fjord, Denmark*, 1914—1919. *Decade Investigations.*

Each figure represents 100 individuals (average of 10 decade averages).

Average of population about 113·20.

114·09	113·34	113·42	112·71	113·85
113·51	113·77	112·41	113·83	113·63
112·65	113·69	113·17	113·55	112·77
112·54	113·04	113·56	113·65	113·03
113·00	113·34	112·59	114·17	112·62
112·82	113·55	113·13	113·96	112·75
112·82	113·36	113·52	112·75	113·25
113·65	114·08	112·70	113·49	112·38
113·12	113·69	113·05	112·44	113·34
113·39	112·28	112·74	113·45	113·52
113·59	112·95	112·98	113·45	112·99
113·12	112·82	112·59	113·24	113·23
112·74	112·45	112·91	113·31	113·47
113·48	112·33	113·05	113·42	113·40
114·07	113·38	113·11	113·56	112·68
113·93	112·94	113·43	113·22	
113·22	113·73	113·86	114·08	
112·70	112·86	112·76	112·93	

TABLE IV.

Station 31, *Ise Fjord, Denmark*, 1914—1919. *Decade Investigations.*

Number of vertebrae in 857 mothers as compared with average number of vertebrae in their offspring (857 decades). Average of population about 113·20.

Maternal number of vertebrae	Frequency of mothers or decades	Average number of vertebrae in offspring
119	3	116·300
118	6	115·617
117	31	114·794
116	65	114·155
115	109	113·851
114	151	113·515
113	185	113·121
112	135	112·948
111	105	112·523
110	47	111·596
109	11	111·245
108	7	110·400
107	2	109·250

TABLE V.

Transplantation of Population Samples from Station 31 *and Station* 34, 1916. *Decade Investigations.*

	Station 31	Station 34
Average number of vertebrae in offspring of transplanted sample	*114·819* ± 1·059 (27 decades)	*108·283* ± 1·363 (11 decades)
Average number of vertebrae in transplanted mothers	113·115 (26 specimens)	107·00 (9 specimens)

TABLE VI.

Station 31, *Ise Fjord, Denmark. Transplantation Experiments*, 1916—1917. *Decade Investigations.*

Average number of vertebrae in offspring of transplanted specimens and in offspring developed under natural conditions.

Maternal number of vertebrae	Average number of vertebrae in offspring of: Transplanted specimens	Not transplanted specimens
116	116·14 (7)	114·14 (57)
115	115·22 (17)	113·86 (94)
114	114·59 (18)	113·51 (136)
113	115·34 (17)	113·12 (166)
112	114·14 (18)	112·95 (123)
111	113·91 (22)	112·54 (86)
110	112·59 (9)	111·58 (41)

The figures in brackets indicate the number of mothers or decades.

TABLE VII.

The Zoarces *Populations of the Ise Fjord—Roskilde Fjord Area.*
Population Analyses 1919.

Average number of vertebrae.

Station 68, Kattegat (outside fjord)	...	117·330
,, 69 (mouth of fjord)	... ...	114·563
,, 70 (mouth of fjord)	... ...	113·732

Station 31,	Ise Fjord	113·2 (1914—19)	Station 71,	Roskilde Fjord	111·168
,, 32,	,,	112·939	,, 72,	,,	110·892
,, 74,	,,	112·243	,, 73,	,,	109·152
,, 75,	,,	112·225	,, 34,	,,	108·0 (1914—19)

INHERITANCE OF CERTAIN CHARACTERS IN THE COWPEA (*VIGNA SINENSIS*). II.

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Introduction.

In the first part of this study (1919), the present writer has recorded the results of experiments on the inheritance in cowpeas of certain factors affecting flower colour, and colour and pattern of the seed coat. The present paper forms a further contribution towards our knowledge of the genetics of the cowpea.

During the progress of the work an insect pest, probably the cowpea weevil (*Chalcodermus annuus*), hitherto unrecorded in St Vincent, made its appearance. The ravages of this pest were so great that most of the seed from several of the F_1 and F_2 cultures was destroyed. For this reason the number of plants in some of the F_2 cultures is disappointingly small.

The experiments described below concern themselves with the following characters:

1. Anthocyanin in stem and leaf stalk.
2. Colour of seed coat pattern.
3. Pod colour.
4. Flower colour.

The Experimental Results.

1. *Anthocyanin in stem and leaf stalk.*

In many varieties of cowpea, the region near the junction of the main stem and petioles is dark red in colour, owing to the presence of anthocyanin pigment in the epidermal cells. The same pigment is also found at the junction of the pinnae with the leaf stalk. In other

varieties pigmentation is totally absent. In crosses between pigmented and unpigmented kinds the following results were obtained :

The F_1. Fully pigmented.

The F_2. Five families were grown, and segregation occurred into the parental types in the following proportions:

Plants	Pigmented	Unpigmented
171	132	39
Expected ...	137	43

This ratio is close to the expected 3 : 1, characteristic of a single genetic difference. Several families were grown in F_3.

The F_3. Of fifteen families grown from F_2 pigmented plants, four families threw pigmented only. There were 91 plants in all. The remaining eleven families segregated into pigmented and unpigmented, the results being:

Plants	Pigmented	Unpigmented
369	276	93
Expected ...	277	92

The ratio of pigmented to unpigmented in segregating families is thus close to 3 : 1.

From F_2 unpigmented plants four families were grown in F_3. All bred true to the unpigmented condition, the number of plants being 140.

Conclusion.

The presence of anthocyanin colouration in the stem and leaf stalk of the cowpea is due to a single unit factor **X**, dominant to its absence.

2. *The Colour of the Seed Coat Pattern.*

In the first part of this study (1919) the present writer established the existence of three Mendelian factors affecting the colour of the seed coat pattern. These are respectively **B** (black), **N** (buff), and **M** (maroon). The genetic composition of the types previously worked with appears to be as follows :

Black	...	**BNM**
Brown	...	**bNM**
Buff	...	**bNm**
Maroon	...	**bnM**
Red	...	**bnm**

The two crosses previously studied were brown by red (**bNM** × **bnm**), giving in F_2, 12 brown and buff, 3 maroon, and 1 red, and black by brown (**BNM** × **bNM**), giving 3 black to 1 brown in F_2. The particular black used in this experiment must have contained the factor *M*, for

when crossed with red, blacks, browns, maroons, and reds, appeared in F_2.

(*a*) *The cross Red by White.*

The red type used in the foregoing experiments was crossed with the white (cream) seeded variety Para. The following were the results:

The F_1. The colour of the seeds of the F_1 plants was exactly the same as the buff type **bNm**, alluded to above.

The F_2. Segregation occurred into buff, red, and white. Unfortunately only 18 plants produced seeds, but it will be seen that the ratio of the three colours was close to 9 : 3 : 4.

Plants	Buff	Red	White
18	11	3	4
Expected ...	10	3	5

The F_3. Sixteen families were grown in F_3. Five of the buffs segregated into buff, red and white in the 9 : 3 : 4 ratio:

Plants	Buff	Red	White
97	49	21	27
Expected ...	55	18	24

Four of the buffs threw buff and red approximately in the 3 : 1 ratio:

Plants	Buff	Red
80	62	18
Expected ...	60	20

Two of the buffs threw buff and white in approximately the 3 : 1 ratio:

Plants	Buff	White
112	78	25
Expected ...	84	28

Of the two reds grown in F_3, one segregated into 9 red and 3 white. The other gave a ratio of 15 buff, 13 red, and 12 white. Natural crossing probably caused this anomalous result.

Three whites threw whites only in F_3, the number of plants in the three families being respectively 96, 16, and 20.

Interpretation of the cross Red by White.

The results of this cross may be explained by assuming that two factors are concerned:

R the factor for a red colour of the seed coat pattern.

N the factor for buff. This factor is without visible effect in the absence of **R**.

The cross red by white may be represented as **nR × Nr**, the types appearing in F_2 being:

9 NR	3 nR	3 Nr	1 nr
Buff	Red	White	White

(*b*) *The cross Black by White.*

A cross between a black and the white variety Para gave F_1 black. In F_2 segregation occurred into black, buff, and white in proportions approximating to a ratio of 9 : 3 : 4. The numerical results from three families were:

	Plants	Black	Buff	White
	95	50	21	24
Expected ...		53	18	24

No F_3 families were grown, but the F_2 data, together with the information acquired from the cross of red by white, enable a satisfactory explanation of the results to be made.

Two factors are concerned in the cross black by white.

R the factor for red.

B the factor for black, which is without visible effect except in presence of **R**.

The factor for buff, **N**, is common to both parents. The cross may be represented thus: black (**BNR**) by white (**bNr**), the types appearing in F_2 being:

9 BNR	3 BNr	3 bNR	1 bNr
Black	White	Buff	White

From the results of this experiment it will be seen that factor **M** is not necessary for the production of black.

(*c*) *The cross Black by Buff.*

The plants of the F_1 showed complete dominance of black. Some of the seeds were slightly mottled, and in these the ground colour was seen to be buff.

The F_2. The results of the F_2 are set forth below. Segregation occurred into black, buff, and red, in proportions suggesting the 12 : 3 : 1 ratio, though the fit is not very close.

	Plants	Black	Buff	Red
	841	644	129	68
Expected ...		632	158	53

The F_3.

1. 17 blacks bred true (480 plants).

2. 16 blacks segregated into black and buff in approximately the 3 : 1 ratio.

Plants	Black	Buff
440	348	92[1]
Expected ...	330	110

3. 14 blacks segregated into black and red in approximately the 3 : 1 ratio.

Plants	Black	Red
382	284	98
Expected ...	287	96

4. 21 blacks segregated into black, buff, and red in approximately the 12 : 3 : 1 ratio.

Plants	Black	Buff	Red
826	612	159	55
Expected ...	619	155	52

5. 8 buffs bred true (307 plants).

6. 19 buffs segregated into buff and red in the 3 : 1 ratio.

Plants	Buff	Red
636	462	174
Expected ...	477	159

7. 11 reds bred true (420 plants).

Interpretation of the cross Black by Buff.

Two factors are involved in this cross, **B**, the factor for black, and **N** the factor for buff. The factor **R** is common to both parents. The cross is of the nature black (**BnR**) by buff (**bNR**). The types appearing in F_2 are as follows:

9 BNR	3 BnR	3 bNR	1 bnR
Black	Black	Buff	Red

The results of this cross demonstrate that factors **B** and **N** are probably independently inherited, and that **N** is not essential for the full production of black.

It will be seen from the above summarised results that the ratios obtained go far to confirm the hypothesis that two independently inherited factors, **B** and **N**, are concerned in the cross black by buff.

[1] These numbers are somewhat far removed from expectation. In two families, Nos. 51 and 105, the number of buffs is abnormally small, being 2 out of 57, and 2 out of 47 respectively. It is possible that accidental mixture of seed has taken place. If these two families are not counted, the ratio of black to buff is 248 : 88, with expectation 252 : 84 on a 3 : 1 basis.

Significance of the Mottling.

It was pointed out above that some of the F_1 seeds showed a certain degree of mottling, and that the buff groundwork was visible in patches in these seeds. In the F_2 blacks, some of the plants showed no mottling at all, whilst of the others some showed red and others buff mottling. Eighteen blacks with no mottling were grown in F_3, and all bred true to black. Fifteen red-mottled blacks were grown in F_3, and all except one segregated into black and red. Many of the blacks were mottled red. Forty-one families were grown from buff mottled blacks. Of these sixteen segregated into black and buff, and twenty-two into black, buff, and red. Three bred true. It may be concluded that mottling indicates that factor B is present in a heterozygous condition, but that some plants showing slightly mottled seeds may be pure for this factor.

(*d*) *The cross New Era by White.*

New Era is one of the best known varieties of cowpea, being highly valued by the farmers of the Southern States as a forage crop. The seeds are thickly and uniformly dotted with a dark blue anthocyanin pigment, on a buff background. The New Era pattern is always associated with purple colouration of the tip of the young pod, calyx, and peduncle.

The F_1 of the cross between New Era and the white variety Para had seeds with the characteristic New Era pattern but with a less intense dotting. In F_2, segregation occurred into New Era, buff, and white approximately in the 9 : 3 : 4 ratio.

	Plants	New Era	Buff	White
	17	10	2	5
Expected ...		10	3	4

In F_3 three families were grown. One of these was from a white and bred true (23 plants). The other two were from plants of the New Era type ; both segregated into New Era, buff, and white in the 9 : 3 : 4 ratio, as follows :

	Plants	New Era	Buff	White
	30	14	6	10
	24	11	4	9
Totals ...	54	25	10	19
Expected ...	—	30	10	14

Interpretation of the results of the cross New Era by White.

The results obtained in the cross New Era by white indicate that two factors are involved :

R the factor for red.

E the factor for New Era pattern on the seed coat, also producing purple colouration of the tip of the young pod, and in the calyx and peduncle. This factor is without effect in the absence of **R**.

The factor for buff, **N**, is common to both parents. The cross is thus New Era (**ENR**) by white (**eNr**), and the types appearing in F_2 are:

9 ENR	3 eNR	3 ENr	1 eNr
New Era	Buff	White	White

It is recognised that the number of plants on which these conclusions are based is very small, and it is intended to repeat the cross in order to obtain a larger series of numbers.

(*e*) *The cross New Era by Black.*

The factors **B** (black), and **E** (New Era), both produce as one of their effects anthocyanin colouration of the tip of the young pod, calyx, and peduncle, though **B** produces a more intense colouration than **E**. It has been shown that both these factors are allelomorphic to their absences. Thus the cross black (**BNR**) by buff (**bNR**) will give in F_2 the ratio 3 black to 1 buff, while the cross New Era (**ENR**) by buff (**eNR**) will give in F_2, 3 New Era to 1 buff. If the factors **B** and **E** are inherited independently, a cross between black and New Era should produce the double recessive, buff, once in sixteen times in F_2, thus:

9 BENR	3 BeNR	3 bENR	1 beNR
?	Black	New Era	Buff

The actual results obtained in the cross black by New Era may be set forth as follows:

The F_1. Black.

The F_2. In F_2 were obtained 73 black and 24 New Era. These results approximate to the 3 : 1 ratio.

Another cross between black and New Era gave the following results:

The F_1. Black.

The F_2. Segregation took place into (*a*) black, (*b*) buff New Era, (*c*) red New Era.

	Plants	Black	Buff New Era	Red New Era
	841	644	129	68
			197	
Ratio ...	3·3	:	1·0	

The presence of both red and buff New Era need not concern us at this point. The important point to note is that the double recessive, **be**, does not occur.

The F_3.

1. Black bred true in 17 families containing 480 plants.

2. In 51 families black segregated into black and New Era in the 3 : 1 ratio.

	Plants		Black		New Era
	1648		1244		404
Expected		...	1236		412
Ratio		...	3·1	:	1·0

3. New Era bred true in 38 families containing 1363 plants.

From the results of these experiments it may be concluded that:

(*a*) **B** and **E** are not inherited independently.

(*b*) **B** and **E** behave as though they were allelomorphic to each other, but it need only be assumed at present that these two factors show repulsion probably on a basis higher than 1 : 15.

(*f*) *The cross Para (albino) by Black-eye.*

This experiment was designed to show whether either or both of the two pattern factors **D** and **H** are present in Para. Since the seed coat of Para is unpigmented no pattern factor will have visible expression.

The F_1. Black solid.

The F_2. Segregation took place in the coloured types into Solid, Watson, Holstein, and Small-eye in approximately the 9 : 3 : 3 : 1 ratio, thus:

	Solid	Watson	Holstein	Small-eye
	DH	**Dh**	**dH**	**dh**
	24	8	3	1
Expected	22	7	7	2

It is clear that Para contains both **D** and **H**. These factors have no visible expression except in presence of the pigment factor **R**, brought in by the black parent.

3. *Pod Colour.*

In certain varieties of cowpea the pod is green in the early stages but shortly before maturity it develops anthocyanin pigment throughout, turning through various shades of colour until when almost ripe it is of a deep purplish-red colour. In purple podded plants there is also colouration of the tip of the young pod, calyx, and peduncle, similar to that caused by the factors **B** (black), and **E** (New Era).

In crosses of purple pod with non-purple pod, the F_1 is purple podded, though less intensely than the parent type. In F_2 segregation occurs into purple and non-purple as follows:

Family	Purple	Non-purple
5*g*	89	30
5—1	76	22
5—3	19	5
5—5	48	11
5—6	14	4
5—8	111	21
5—9	30	5
5—9*a*	42	11
5—9*b*	20	6
Totals ...	449	115
Expected ...	423	141
Ratio ...	3·9 :	1·0

This ratio is somewhat far removed from the simple 3 : 1 type, and for this reason the F_2 families have been presented separately.

The F_3.

1. 7 purples segregated into purple and non-purple.

Family	Purple	Non-purple
1	31	8
2	27	6
3	28	8
4	23	5
5	23	4
6	22	4
7	19	8
Totals ...	173	43
Expected...	162	54
Ratio ...	5·0 :	1·0

2. 2 purples bred true (119 plants).

3. 4 non-purples bred true (89 plants).

The peculiar feature of these results is the ratio 3·9 : 1·0 of purple to non-purple in F_2, and the very similar ratio of 4·0 : 1·0 in heterozygous F_3 families. It is evident that a much larger series of numbers is required before the exact mode of inheritance of purple pod is elucidated. It may be assumed meanwhile that one main factor, P, is responsible for purple pod.

Relation between the factors B (*black*), E (*New Era*), *and* P (*purple pod*).

The interesting feature of the three factors B, E, and P, is that any of them may produce anthocyanin pigmentation of the young pod, calyx, and peduncle. E causes a lesser development of colour than B, and B slightly less than P.

The relation of **B** and **E** has already been dealt with, and it was shown that these two factors repel each other in gametogenesis. The relation between the factors **B** and **P** will now be discussed.

The cross Black (**Bp**) *by Purple Pod* (**bP**).

The F_1. The F_1 possessed black seeds and purple pods, though the colour of the pod was less intense than that of the purple podded parent.

The F_2. In the F_2 three types appeared, viz. **BP**, **Bp**, and **bP**. Classifying the F_2 the results were:

Plants	BP	Bp	bP
273	168	40	65

Taking the ratio of **B** to **b** we obtain 208 : 65 (expectation 205 : 68), while the ratio of **P** to **p** is 233 : 40 (expectation 205 : 68).

The F_3.

1. Progeny of black, purple-podded F_2 plants.

Families	Plants	BP	Bp	bP
14	556	305	129	122

Here the ratio of **B** to **b** is 434 : 122, and **P** to **p** is 427 : 129. The expectation in both cases on a 3 : 1 basis is 417 : 139.

2. Progeny of non-black, purple podded F_2 plants.

Families	Plants	BP	Bp	bP
23	1036	0	0	1036

3. Progeny of black, non-purple podded F_2 plants.

Families	Plants	BP	Bp	bP
5	181	0	181	0

The main conclusions which may be arrived at from a survey of these results are:

1. The ratio of black to non-black in both F_2 and F_3 cultures is close to 3 : 1, confirming the conclusion previously arrived at that black is allelomorphic to its absence.

2. The ratio of purple pod to non-purple pod in F_2 is somewhat far removed from 3 : 1, being 5·9 : 1·0, although in the segregating F_3 families the 3 : 1 ratio is more closely followed. Thus it is not certain that purple pod is due entirely to a single factor **P**. This conclusion was arrived at from a study of other crosses.

3. The non-appearance of the double recessive non-black, non-purple pod in the F_2 and F_3 cultures of the cross **Bp** by **bP**, indicates that these two factors are repelled on a basis probably higher than 7 : 1.

On the chromosome hypothesis of Morgan *et al.* (1915), the factors B, P, and E, are located in the same chromosome. The evidence is not strong enough to say that complete repulsion occurs between B and E, and between B and P; the factors are, however, so near together that crossing over seldom occurs.

If these three factors could be shown to occupy identical loci, they would then constitute a triple series of multiple allelomorphs. In nearly all cases in which multiple allelomorphs have been discovered, the factor-differences produce similar effects. The fact that B, P, and E, all produce colouration of young pod, calyx, and peduncle as one of their effects is an argument in favour of the multiple allelomorph theory.

4. *Flower Colour.*

In the F_2 of a cross between red (dark flower), and Para (white flower), it was expected that segregation would occur into 3 dark and 1 white. Some of the apparently white flowers were found on close examination to possess a minute degree of anthocyanin pigmentation in the wings. This cannot usually be seen with the naked eye, and is best detected by dipping the flower in dilute hydrochloric acid, when a pink flush appears if pigmentation be present. This type of flower colour may conveniently be termed "tinged." The proportions in which dark, tinged, and white, occurred in the cross red by Para were:

Plants	Dark	Tinged	White
18	14	3	1

The F_3 results were as follows:

1. 3 darks segregated into dark, tinged, and white.

	Plants	Dark	Tinged	White
	118	95	19	4
	46	35	10	1
	46	34	10	2
Totals	210	164	39	7
Expectation on 12 : 3 : 1 basis	—	157	39	13

2. 7 darks segregated into dark and tinged in the 3 : 1 ratio.

Plants	Dark	Tinged
94	73	21
Expected ...	71	23

3. 3 darks bred true (76 plants).

4. 2 tinged segregated into tinged and white in the 3 : 1 ratio.

Plants	Tinged	White
116	90	26
Expected ...	87	29

5. 1 tinged bred true (16 plants).

Interpretation of the Results.

The evidence, although incomplete, indicates that the tinged type of flower is due to a factor which may be called **G**. This factor is recessive to **D**, the factor for dark flower, and tinged and white form a pair of allelomorphic characters.

It was thought at first that the cross was of the nature **DGR** × **Dgr** giving in F_2:

9 DGR	3 DgR	3 dGr	1 dgr
Dark	Dark	Tinged	White

or altogether 12 dark, 3 tinged, and 1 white. According to this hypothesis the number of darks segregating into dark and tinged, and dark and white respectively in F_3 should be equal in number. Actually no darks segregated into dark and white, whereas 7 segregated into dark and tinged. The F_3 families which segregate into all three types should exhibit the 12 : 3 : 1 ratio. The actual figures show considerable deficiency in the number of whites. It may be noted that it is not easy to distinguish between tinged and white in some plants, and possibly some plants have been classified as tinged which are really white.

On the whole the evidence is in favour of the view that the cross is **DGR** by **Dgr**, but a much larger series of numbers is necessary to substantiate the theory.

The two factors **D** and **L** have no visible effect except in presence of the factor **R** (red). The factor **G** produces the tinged type of flower in plants with white seeds.

General Summary.

1. The presence of anthocyanin colouration in stem and leaf stalk is due to a factor **X**, dominant to its absence.

2. The colours of the seed coat pattern, black, brown, buff, maroon, red and white may be regarded as due to various combinations of four factors, **B** (black), **N** (buff), **M** (maroon), and **R** (red), thus:

Black	...	BNMR
,,	...	BNmR
,,	...	BnmR
,,	...	BnMR
Brown	...	bNMR
Buff	...	bNmR
Maroon	...	bnMR
Red	...	bnmR
White	...	due to absence of R

3. The New Era pattern of the seed coat is due to a factor **E**, which has no visible effect in absence of **R**.

4. The albino type Para carries the two seed coat pattern factors **D** and **H** (either H_1 or H_2). They produce no visible effect except in presence of **R**.

5. The purple colour of the pod is due to one main factor **P**. The marked deficiency in the expected number of recessives leads to the view that more than one factor may be involved.

6. The factors **B** (black), and **E** (New Era), show repulsion on a basis probably higher than 1 : 15.

7. The factors **B** (black), and **P** (purple pod), show repulsion, probably on a basis higher than 1 : 7.

8. The type of flower colour known as tinged is due to a factor **G**, dominant to its absence, but recessive to **D**, the factor for dark flower.

REFERENCES.

HARLAND, S. C. 1919. "Inheritance of certain characters in the cowpea (*Vigna sinensis*)." *Journ. of Genetics*, Vol. VIII. No. 2.

MORGAN, T. H., STURTEVANT, A. H., MULLER, H. J., and BRIDGES, C. B. 1915. *The Mechanism of Mendelian Heredity.*

INHERITANCE IN *RICINUS COMMUNIS*, L. PART I.

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Introduction.

This paper presents data on the mode of inheritance of certain characters in the Castor-oil plant, *Ricinus communis*, L. As pointed out by White (1918 a), *Ricinus* is a good subject for genetic experiments. It exists in a large number of distinct and well marked varieties which readily intercross. The seeds remain viable for several years. Furthermore, back-crosses, which are so necessary for the proper study of linkage phenomena, are easily effected.

The characters which have been studied are:

1. Bloom.
2. Spines on the capsule.
3. Colour of the vegetative parts.

1. *Bloom.*

Varieties of *Ricinus* are distinguished by the presence or absence on the plant body of a waxy bloom. In varieties with bloom two main types exist:

A. Bloom on stem, petioles, and capsules.

B. Bloom on stem, petioles, capsules, and also on the under-surface of the leaf.

In class A one or more sub-types may be recognised, but so far no attention has been given to these. Type A is the only one which has been used in the experiments.

Previous Investigations.

Dr O. E. White (1918b) deals with the mode of inheritance of bloom. He states:

> Crosses of bloom × no-bloom give either complete or partial dominance of bloom in F_1. In F_2, approximately 3 with bloom : 1 no-bloom were obtained (actual

numbers being 1108 bloom : 377 no-bloom). In F_3, seeds from unguarded F_2 plants without bloom produced only plants without bloom. Seeds from unguarded F_2 plants with bloom either bred true in F_3 or gave 3 bloom : 1 no-bloom families. In one cross of bloom × no-bloom, the F_1 plants had bloom, but were only lightly covered as compared with their "bloom" parent. In F_2, the plants approximated a ratio of 3 bloom : 1 no-bloom as usual, though many of those with bloom were lightly covered as in F_1.

The present writer (1918), in a short preliminary note, put forward the results of one cross between bloom and no-bloom. The F_1 was intermediate and in F_2 331 bloom and 118 no-bloom were obtained. From these results it was considered probable that the presence and absence of bloom in *Ricinus* constitute a Mendelian pair of characters.

The Experimental Results.

Further results are presented below on the mode of inheritance of bloom, in crosses of bloom × no-bloom.

The cross St Vincent Native Type 2 × *Ricinus communis* var. *Gibsoni*.

Bloom **No-bloom**

The F_1. Fourteen plants were grown. All possessed bloom, but were lightly covered in comparison with the bloom parent.

The F_2. Families were grown from all the fourteen F_1 plants. Segregation took place into bloom and no-bloom. During the examination it was established that some plants could not be classified correctly until the fruiting stage, as bloom was only present on the stem of the flowering spikes. To distinguish between the various types of bloom proved to be difficult in practice, and on this account the plants were divided only into the two broad classes, bloom and no-bloom.

Family	Bloom	No-bloom
1	27	6
2	42	16
3	55	12
4	48	10
5	69	13
6	109	23
7	26	9
8	75	25
9	67	17
10	54	15
11	21	8
12	55	9
13	35	15
14	33	10
Totals ...	716	188
Expected ...	678	226

The F_3. Of 21 families grown from F_2 plants with bloom, 10 bred true (230 plants).

11 families segregated into bloom and no-bloom.

Family	Bloom	No-bloom
2—22	26	11
3—20	63	19
3—31	16	4
6—25	33	18
8—21	39	12
9— 7	65	16
9—16	12	6
9—26	26	12
3— 1	2	3
6—28	13	6
8—24	5	1
Totals ...	300	108
Expected ...	306	102

Of 10 families grown from F_2 plants with no-bloom, all bred true (217 plants).

Back crosses of F_1 plants with the no-bloom parent.

Cross	Bloom	No-bloom
1	12	18
2	10	9
3	2	1
4	8	7
5	23	17
6	7	2
7	2	5
8	12	7
9	3	10
10	28	18
11	3	4
12	4	5
13	5	6
14	0	1
15	1	2
16	4	5
17	1	5
18	2	1
19	1	4
20	8	11
21	5	3
22	8	4
23	6	10
24	9	11
25	0	2
Totals ...	164	168
Expected ...	166	166

The conclusion previously arrived at by White and the present writer that presence and absence of bloom form a simple Mendelian pair of characters is confirmed by the above results. The symbols **B** and **b** will be used to denote presence and absence of bloom respectively. It will be noted that there is a marked excess of dominants in the F_2

families but there is no marked deviation from expectation in F_3 families or in the back-crosses.

2. *Spines on the Capsule.*

A variety of *Ricinus* devoid of spines on the capsule was collected in the Grenadines. It bred true on being grown in pedigree culture. Grisebach (1864) records this variety under the name *inermis*.

In a cross between spiny and spineless the following results were obtained:

The F_1. The fourteen F_1 plants were spiny, though the number of spines per capsule was obviously less than in the spiny parent. Spininess is therefore incompletely dominant.

The F_2. Families were grown from all the F_1 plants. The results are presented below.

Family	Spiny	Spineless
1	21	2
2	38	19
3	37	22
4	32	13
5	40	18
6	99	22
7	23	12
8	71	27
9	62	18
10	45	24
11	19	9
12	43	19
13	29	21
14	33	9
Totals ...	592	235
Expected ...	620	207
Ratio ...	2·5 :	1·0

The F_3. Of 17 families grown from F_2 plants which were spiny, 5 bred true (219 plants).

Twelve families segretated into spiny and spineless.

Family	Spiny	Spineless
3—20	29	7
3—30	56	11
6—25	45	6
6—27	32	17
6—34	29	14
8—21	37	8
9— 7	49	20
9—15	29	10
9—16	15	3
9—25	10	2
13— 9	31	6
13—10	7	5
Totals ...	369	109
Expected ...	359	120
Ratio ...	3·4 :	1·0

Three families were grown from F_2 spineless plants. All bred true (67 plants).

Back crosses of $F_1 \times$ Spineless.

Cross	Spiny	Spineless
1	55	58
2	12	8
3	46	50
4	31	30
5	68	44
6	45	34
7	41	52
Totals ...	298	276
Expected ...	287	287

Conclusion.

The above results indicate that the presence and absence of spines on the capsule are a simple pair of Mendelian characters. The factor for spines may be denoted by **S**, and its allelomorph by **s**.

3. *Colour of the Vegetative Parts.*

According to the observations of White (1918b), stem colour in *Ricinus* may be divided into five categories—bright green, green with reddish blush on sunny side, carmine or rose red, mahogany red, and purple (dark red). This author also states:

> Associated with the types of stem colouring are pigmented areas in other parts of the plant. The mahogany red stemmed plants have mahogany red leaves and fruits. The rose and red blush stemmed types have green leaves with red or reddish midribs. The dark purplish-red (mahogany bloom) stemmed plants have dark purplish-red leaves and fruits.

His experiments may be summarised thus:

1. Red-blush and green form an allelomorphic pair of characters.
2. Red-blush and mahogany are allelomorphic.
3. Rose and red-blush are allelomorphic.

The results of the present writer's experiments will now be given.

1. *The Cross Red-blush × Mahogany.*

This cross gave results similar to those obtained by White. The F_1 was rose or carmine stemmed, and in F_2 segregation occurred into 102 rose and red-blush, and 31 mahogany (expectation 103:34).

The F_3. A few families were grown in F_3.

Of 7 families grown from rose and red-blush F_2 parents, 3 bred true (25 plants).

The remaining families segregated into rose and red-blush, and mahogany.

Family	Rose and red-blush	Mahogany
1	8	2
2	7	1
3	5	4
4	12	3
Totals ...	32	10
Expected ...	32	11

Of 4 families grown from mahogany F_2 plants, 3 bred true (34 plants), while the other consisted of 15 mahogany and 3 rose and red-blush. The parent of this family was self-fertilised, but it is possible that accidental admixture of seed may have taken place.

The back-cross F_1 × mahogany.

A single back cross was made and produced seeds giving rise to 37 rose and 43 mahogany (expectation 40:40).

Conclusion.

Red-blush and mahogany are an allelomorphic pair of characters.

2. *The Cross Green × Mahogany.*

The F_1. Fourteen plants were grown. The stem colour was rose.

The F_2. Some difficulty was experienced in classifying the plants. Green and mahogany could be distinguished easily, but there appeared to be several shades of rose, grading down to red-blush. A new type was recorded which was called "tinged." The young leaves are strongly reddened, but the colour disappears as the leaves unfold, so that old leaves are practically green. The glands on the leaf stalk retain their colour, and a faint amount of colour is seen in the stem.

In an F_2 population some confusion is likely to arise in classifying the plants, owing to the fact that it is not always easy to distinguish between some of the paler types of red-blush and tinged. In practice the various shades of rose and tinged were grouped together. Grouping the plants into (*a*) rose and tinged, (*b*) mahogany, and (*c*) green the following results were obtained:

Family	Rose and Tinged	Green	Mahogany
1	19	9	9
2	41	12	6
3	44	14	13
4	37	12	14
5	64	15	22
6	98	38	32
7	75	25	16
8	93	25	26
9	61	27	16
10	59	13	19
11	27	3	8
12	50	18	8
13	56	20	14
14	43	15	12
Totals	767	246	215
Expectation on 10 : 3 : 3 basis	767	230	230

The F_3.

Family	F_2 parent	Rose	Mahogany	Green	Tinged
3—20	Rose	45	18	20	2
3—31	,,	10	1	7	2
6—34	,,	33	8	5	1
8—21	,,	33	6	12	1
9—16	,,	11	3	4	0
9—25	,,	9	3	—	—
13— 9	,,	30	7	—	—
3—30	,,	56	—	14	—
6—28	,,	37	—	15	—
9—26	,,	29	—	8	—
6—27	,,	49	—	—	—
9—15	,,	39	—	—	—
2—22	Mahogany	—	24	—	13
9— 7	,,	—	59	—	21
3—26	Green	—	—	7	1
8—16	,,	—	—	9	4
9—18	,,	—	—	30	9
13—16	,,	—	—	53	14
7— 7	Tinged	—	—	—	22
13—10	,,	—	—	1*	11

* Probable vicinist.

From these results it will be seen that rose breeds true in 2 families containing 88 plants, and segregates into rose, green, tinged, and mahogany in 5 families. Remembering the difficulty of distinguishing between rose and tinged, and classifying the plants into rose and tinged, green and mahogany, we obtain the ratio 138 : 36 : 48, with expectation 139 : 41 : 41 on a 10 : 3 : 3 basis. Rose segregates into rose and mahogany in 2 families, in the proportion of 39 rose : 10 mahogany (expectation 37 : 12 on a 3 : 1 basis). Rose segregates into rose and green in 3 families, in the proportion of 122 rose to 37 green (expectation 119 : 40 on a 3 : 1 basis).

Two families from mahogany F_2 plants segregate into mahogany and tinged in the ratio 83 : 34 (expectation 88 : 29 on a 3 : 1 basis).

Four families from green F_2 plants segregate into green and tinged, 99 : 28 (expectation 95 : 32 on a 3 : 1 basis).

Two families from F_2 plants breed true (33 plants). One family contained a green plant, which is ascribed to vicinism.

Back-crosses.

Back-crosses were made of F_1 with green, and F_1 with mahogany. The results are set forth below:

(*a*) *The Back-cross* F_1 × *Green.*

Cross	Rose	Green
1	70	67
2	35	31
3	50	45
4	19	44
5	54	59
6	45	37
7	60	41
Totals ...	333	324
Expected...	329	329

(*b*) *The back-cross* F_1 × *Mahogany.*

Cross	Rose	Mahogany
1	16	16
2	14	13
3	3	2
4	7	8
5	28	22
6	5	5
7	6	11
8	12	7
9	14	13
10	39	41
11	3	4
12	3	0
13	6	3
14	4	7
15	0	1
16	2	1
17	4	5
18	2	4
19	1	2
20	2	3
21	10	9
22	5	3
23	3	9
24	12	4
25	12	7
26	1	3
Totals ...	214	203
Expected...	209	209

Interpretation of the Experimental Results.

The results of the cross green × mahogany may be explained by the assumption that two independently inherited factor differences are concerned. The cross may be represented thus:

Parents	Green × Mahogany			
	mG	Mg		
	Rose			
F_1	Mm Gg			
F_2	9 Rose	3 Green	3 Mahogany	1 Tinged
	MG	mG	Mg	mg

It has been stated that difficulty is often experienced in distinguishing tinged from some of the shades of rose, and therefore in classification rose and tinged have been combined.

It remains now to consider to what extent the hypothesis is supported by the experimental results.

1. The expected F_2 ratio of 10 rose and tinged : 3 green : 3 mahogany is closely approximated to in the actual results.

2. Plants which were rose in F_2 should exhibit four types of behaviour in F_3:

(*a*) Breed true.

(*b*) Segregate into rose and tinged, green, and mahogany in the 10 : 3 : 3 ratio.

(*c*) Segregate into rose and mahogany in the 3 : 1 ratio.

(*d*) Segregate into rose and green in the 3 : 1 ratio.

All these types of behaviour are demonstrated in the experimental results, and the observed and expected ratios agree closely.

3. Mahogany F_2 plants should in F_3 either breed true or segregate into mahogany and tinged in the 3 : 1 ratio. No constant mahogany families were observed; both the families grown showed the ratio of 3 mahogany to 1 tinged. Had a larger number of families been grown, some would no doubt have bred true to mahogany.

4. Green F_2 plants should in F_3 either breed true or segregate into 3 green and 1 tinged. Owing probably to the small number of families grown no constant greens occurred, but four families segregated into 3 green and 1 tinged.

5. Tinged F_2 plants should breed true in F_3. This expectation was realised in two families.

6. The back-cross of F_1 × green is of the nature **Mm Gg × mG mG**, which should produce rose and green in the ratio 1 : 1. The observed results agree closely with the expected.

7. The back-cross of F_1 × mahogany is of the nature **Mm Gg × Mg Mg**, which should produce rose and mahogany in the 1 : 1 ratio. The observed results again agree with the expected.

To sum up: the hypothesis that two independently inherited Mendelian factors are concerned in the cross green by mahogany, is confirmed by the experimental results.

The two factors are:

G. The factor for green, which converts tinged into green, and

mahogany into rose. This factor can perhaps be regarded as a colour inhibitor.

M. The factor for mahogany.

Relation between the factors **S** (spines), and **M** (mahogany).

The cross green, bloom, spineless by mahogany, no bloom, spiny, is of the nature **GB sm × gb SM.**

Owing to the difficulty of separating rose (**MG**), from tinged (**mg**), in F_2, it was not possible to study the relation of **S** and **M** in F_2 families of this cross.

From the results of the back-cross of F_1 by double recessive **sm** (green spineless), placed below, it will be seen that the four phenotypes **SM, Sm, sM,** and **sm** occur in the ratio 1 : 1 : 1 : 1.

Back-crosses of **Sm Sm × sm sm.**

Family	SM	Sm	sM	sm
1	28	26	27	31
2	9	3	3	2
3	26	20	24	26
4	6	25	13	18
5	34	33	19	24
6	25	21	18	16
7	24	17	31	21
Totals ...	152	145	135	138
Expected ...	142·5	142·5	142·5	142·5
Ratio ...	1·1	1·0	0·9	1·0

Conclusion.

The factors **S** and **M** are independently inherited.

Relation between the factors **B** (bloom) and **S** (spines).

The relation between these two factors has been studied in a cross between **Bs** and **bS.** The F_2 results of this cross are given below.

Family	BS	Bs	bS	bs
1	17	0	4	2
2	29	11	9	7
3	31	14	6	5
4	25	10	6	3
5	40	15	10	3
6	78	18	20	4
7	44	14	16	4
8	52	19	18	7
9	48	14	13	3
10	33	20	11	4
11	13	7	6	2
12	39	15	4	5
13	18	16	12	4
14	25	7	8	2
Totals ...	492	180	143	55
Expected ...	489	163	163	54

From these results it may be concluded that **B** and **S** are independently inherited.

Relation between the factors **G** (green), and **B** (bloom).

The relation of **G** and **B** was investigated in the back-cross **Gg Bb × gb gb.** The following were the results:

Family	GB	Gb	gB	gb
1	10	6	2	12
2	6	2	4	7
3	1	1	1	0
4	3	4	5	3
5	14	8	9	9
6	3	1	4	1
7	3	1	0	3
8	7	5	5	2
9	1	5	2	5
10	15	7	13	11
11	1	2	2	2
12	1	5	3	0
13	1	3	4	3
14	9	12	8	17
15	16	17	12	12
Totals ...	91	79	74	87
Expected ...	83	83	83	83

It will be seen from these results that **GB** and **gb** are in excess of **Gb** and **gB**. By Morgan's method of calculation the percentage of crossing over between **G** and **B** is 46·2, practically independent inheritance.

With such a small series of numbers it would be unsafe to assume that **G** and **B** are linked, but the results indicate the need for further experiments.

The relation between the factors **M** (mahogany), and **B** (bloom).

In the cross green, bloom, by mahogany, no-bloom, it was noticed that the combination green, no-bloom occurred only once among a large number of F_2 plants. Since the cross is **GB m × gb M** it is clear that the combination **mb**, *i.e.* green, no-bloom, and tinged, no-bloom should occur once in sixteen times if **M** and **B** are independently inherited. The F_2 results are placed below.

Family	BM	Bm	bM	bm
1	76	23	21	1
2	39	18	20	0
3	88	34	33	1
4	208	81	74	0
5	154	44	48	0
Totals ...	565	200	196	2

These results indicate that factors **B** and **M** are repelled, though no attempt will be made to calculate the theoretical gametic series from

the F_2 results. It is hoped to elucidate the relation of the two factors by a series of back-crosses with the double recessive.

General Summary.

This paper is concerned with the mode of inheritance of certain characters in the castor-oil plant, *Ricinus communis*, L. The experimental results may be summarised as follows:

1. The presence of bloom is partially dominant to its absence. Presence and absence of bloom behave as a pair of simple allelomorphic factors. The factor for bloom is denoted by **B**, and its allelomorph by **b**.

2. The presence and absence of spines on the capsule form another pair of allelomorphs, with spininess partially dominant. The factor for spines is denoted by **S**, and its allelomorph by **s**.

3. The experiments on the inheritance of stem colour in the cross green by mahogany indicate that two independently inherited Mendelian factors are involved:

M. The factor for mahogany.

G. The factor for green.

The four types resulting from the cross **Mg** × **mG** are:

9 MG	3 Mg	3 mG	1 mg
Rose	Mahogany	Green	Tinged

4. Experiments on the relation between the different factors show that **S** and **M**, **S** and **B**, **M** and **G**, and perhaps **G** and **B** are independently inherited. Factors **M** and **B** show repulsion in the cross **Mb** × **mB**.

REFERENCES.

Grisebach, H. R. A. 1864. *Flora of the British West Indies.*

Harland, S. C. "Notes on Castor-oil in the West Indies." *Agricultural News*, Barbados, No. 416, p. 100.

White, O. E. 1918 *a*. "Breeding New Castor Beans." *Journal of Heredity*, Vol. ix. No. 5, May—June.

—— *b*. "Inheritance Studies on Castor Beans." *Brooklyn Botanic Garden Memoirs*, i. 513—520.

INHERITANCE IN *DOLICHOS LABLAB*, L. PART I.

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Introduction.

The plant known as the bonavist or hyacinth bean (*Dolichos lablab*, L.), is widely distributed in tropical and sub-tropical countries. The young pods are used as a substitute for French beans, and the beans themselves are by no means unpalatable. When cooked they somewhat resemble Lima beans in flavour.

A large number of varieties have been collected from various West Indian islands, and for other sorts the writer is indebted to the United States Department of Agriculture and Messrs Ryder of St Albans.

Pedigree cultures from single plants invariably breed true, and it is probable that *Dolichos lablab*, like *Pisum*, is naturally self-fertilising. Dehiscence of the anthers takes place at least one, and sometimes two days before the flowers open. Several thousand plants have been used in these experiments, and so far no case of natural crossing has been recorded. The absence of natural crossing is not due to the absence of insect visitors, for the large carpenter bee (*Xylocopa* sp.), which has previously been noted by the writer (1919) as responsible for a considerable amount of natural crossing in the cowpea, visits the flowers extensively.

Waby (1917) considers that his type *Dolichos lablab* var. *macrocarpum* resulted from a natural cross between the two varieties Park Runner and Vilmorin's Stringless. This cannot be regarded as proven, although it is not impossible that occasional natural crosses are effected through the agency of thrips, which at certain times are very abundant in the flowers. The time of dehiscence of the anthers varies somewhat in different sorts, and this observation lends some support to the theory that natural crosses are possible by the transference by insect agency of pollen from an early dehiscing to the stigma of a late dehiscing variety.

The following characters have been studied in these experiments:

1. Habit of growth.
2. Anthocyanin colouration of flower, seed, and vegetative parts.

1. Habit of Growth.

The various races of *Dolichos* may be divided into two main groups, according to whether the growth habit is indeterminate (climbing or determinate, dwarf or bush). Emerson (1916) has described these two types of growth habit in the French bean (*Phaseolus vulgaris*), and he states that they also occur in the Lima bean (*Phaseolus lunatus*). The determinate types are called bush beans from the fact that the main axis terminates in an inflorescence after from 4 to 8 nodes have been developed. The secondary branches which arise from the lower nodes also terminate in flower clusters. The indeterminate types are climbing or pole beans. In these, an indefinite number of nodes is produced by the main axis, termination of growth being due to unfavourable conditions. Emerson (l.c.) records cases of the axis of a pole bean terminating in a flower cluster. This condition has not been seen in *Dolichos*.

The Experimental Results.

Determinate	× Indeterminate
St Vincent Bush White	Vilmorin's Stringless

The F_1. The F_1 consisted of two plants, both of which possessed the indeterminate habit.

The F_2. The number of plants grown was 389. Of these 298 were indeterminate and 91 determinate. The ratio of indeterminate to determinate is close to the simple 3–1 ratio characteristic of a single factor difference. The expected numbers are 292 indeterminate and 97 determinate.

The F_3. Seventeen families were grown from F_2 indeterminates. Four families bred true to indeterminate, consisting altogether of 271 plants. Thirteen families segregated into indeterminate and determinate in the 3–1 ratio. Of 635 plants, 478 were indeterminate and 157 determinate, with expectation 476 and 159 respectively.

Ten families were grown from F_2 determinates. All these families bred true,—containing altogether 325 plants.

The F_4. Twenty-eight families were grown. These were derived from families which bred true to the determinate habit in F_3. These twenty-eight families, containing altogether 829 plants, continued to breed true in F_4.

CONCLUSION.

From these results it may be concluded that the determinate and indeterminate habits of growth in *Dolichos lablab* constitute a simple allelomorphic pair of characters, with complete dominance of indeterminate habit. The factor for indeterminate habit will be denoted by the symbol I.

2. ANTHOCYANIN COLOURATION OF FLOWER, SEED, AND VEGETATIVE PARTS.

The cross which has just been discussed was made primarily with the object of elucidating the mode of inheritance of growth habit. Both parents were white flowered, while the two F_1 plants were purple flowered. The first case of this kind was recorded in Sweet peas, and investigated by Messrs Bateson and Punnett and Miss Saunders (1905). In the Sweet pea, two white parents each contain a factor, which alone is incapable of forming colour. Each of these two factors is independently inherited and thus in F_2 the ratio of coloured individuals to white is 9 : 7. The symbols used to represent the two complementary factors are C and R, their respective allelomorphs being represented by c and r. Miss Saunders (1909) has also shown that in Stocks (*Matthiola*) coloured F_1 may be produced by crossing two types both devoid of sap colour. The ratio between sap-coloured and non-sap-coloured plants in F_2 is approximately 9 : 7. The appearance of the sap-colour is due to the presence of two complementary factors. The case of Antirrhinum, studied by Miss Wheldale (1907), may also be briefly referred to. In this plant the appearance of the red and magenta flower colours is similarly dependent on the presence of two complementary factors. One of these factors produces either a bright yellow or a very pale "ivory" yellow in the flowers, while the other complementary factor may be present in actual whites.

The results of experiments on the inheritance of flower colour in *Dolichos* will now be dealt with.

The Experimental Results.

St Vincent Bush White × Vilmorin's Stringless White.

The F_1. There were two plants, both purple-flowered.

The F_2. Families were grown from both the above plants. The results from the combined families are as follows:

	Purple	White	Ratio
	484	342	9·3 : 6·7
Expected	465	361	

The F_3. The F_3 families from F_2 purples showed three types of behaviour:

(*a*) Bred true (5 families).

(*b*) Segregated into purple and white in the 9 : 7 ratio (35 families).

(*c*) Segregated into purple and white in the 3 : 1 ratio (25 families).

The detailed results are as follows:

(*a*) Breeding true to purple.

Family	Purple	White
1	34	0
2	18	0
3	22	0
4	18	0
5	140	0
Totals ...	232	0

(*b*) Segregating into purple and white in the 9 : 7 ratio.

Family	Purple	White
6	20	11
7	13	10
8	14	12
9	10	8
10	11	13
11	71	72
12	11	9
13	14	10
14	12	10
15	16	9
16	14	11
17	14	14
18	17	13
19	17	14
20	13	12
21	10	12
22	13	13
23	21	13
24	11	7
25	19	13
26	18	12
27	9	9
28	13	7
29	14	18
30	9	9
31	53	33
32	57	34
33	59	38
34	22	13
35	24	22
36	21	15
37	31	20
38	17	18
39	26	20
40	42	37
Totals ...	756	591
Expected ...	758	589

Note. Some of the above families are small, and it is not improbable that a few are really of the 3—1 type of ratio.

(*c*) Segregating into purple and white in the 3 : 1 ratio.

Family	Purple	White
41	19	6
42	20	8
43	13	6
44	12	4
45	18	6
46	17	6
47	20	7
48	13	5
49	16	6
50	8	2
51	19	4
53	22	7
54	13	6
55	18	7
56	78	20
57	90	31
58	17	4
59	11	4
60	28	8
61	42	11
62	49	4
63	36	7
64	24	11
65	80	28
66	40	21
Totals ...	723	229
Expected ...	714	238

In regard to the behaviour of the white flowered plants in F_3 it may be stated briefly that twenty-five families were grown containing 716 plants—all of which were white.

The F_4. Several families were continued into F_4. The results may be set forth as follows:

Series 64. Family 64 threw 24 purples and 11 whites in F_3. Assuming that the type of ratio in this family is 3–1, it is to be expected that of every 3 purples 2 will segregate into purple and white in the 3–1 ratio, and 1 will breed true. The whites will breed true. Of 6 families from F_3 purples, 3 bred true (344 plants), and 3 segregated into purple and white in the 3–1 ratio, the actual numbers being 80 purple and 35 white. From F_3 whites 4 families containing 129 plants were grown. All the plants were white flowered.

Series 63. Family 63 belonged to the 3–1 type of segregation, having thrown 36 purples and 7 whites in F_3. In F_4 sixteen families were grown:

(*a*) Of 12 families from purples, 9 segregated into purples and whites in the 3–1 ratio (99 : 41), while 3 families bred true (58 plants).

(*b*) Of 4 families from whites, all bred true (52 plants).

It is to be regretted that no F_3 families which showed the 9 : 7 type of ratio were continued into F_4.

Interpretation of the Experimental Results.

1. Two factors are concerned in the production of the purple colour of the flower. These may be denoted by the symbols **C** and **R**, while their allelomorphs may be represented by **c** and **r**.

2. In the presence of both factors the flower is purple. In the absence of either the flower is white.

3. According to this view the cross is of the nature **cR** × **Cr** (St Vincent Bush × Vilmorin's Stringless). The genotypes appearing in F_2 will be as follows:

Homozygous			Heterozygous			
Genotype	No.	Colour	Genotype	No.	Colour	Behaviour in F_3
CRCR	1	Purple	CRCr	2	Purple	$3p : 1w$
CrCr	1	White	CRcR	2	Purple	$3p : 1w$
cRcR	1	White	CRcr	4	Purple	$9p : 7w$
crcr	1	White	Crcr	2	White	All white
			cRcr	2	White	All white

The ratio of purple to white in F_2 is 9 : 7. In F_3 of the 9 purples 1 will breed true; of the remaining 8 purples 4 will segregate into purple and white in the 9 : 7 ratio, and 4 into purple and white in the 3 : 1 ratio. The ratio between the three kinds of families is thus 1 : 4 : 4. Of the 7 whites, all will breed true. In F_4 the purples from F_3 families which produced the 3–1 type of ratio, will again produce this type of ratio, or else breed true, and the ratio of pure to heterozygous purples will be 2 : 1. Purples from F_3 families which exhibited the 9 : 7 ratio, will behave in the same way as F_2 purples, i.e. $\frac{1}{9}$ will breed true, $\frac{4}{9}$ will give $3\,p : 1\,w$, and $\frac{4}{9}$ will give $9\,p : 7\,w$. All F_3 whites will remain constant in F_4.

It remains to see how far this hypothesis is confirmed by the experimental results. From the latter we see that:

1. The ratio of purple to white in F_2 is 484 : 342 (9·3 : 6·7), which is near to the expected one of 9 : 7.

2. The 25 F_2 whites which were grown on into F_3, produced only whites. This is in accordance with theory.

3. Of 65 families from F_2 purples, 35 exhibited the 9 : 7 ratio, 25 the 3 : 1 ratio, while 5 bred true. The expected numbers are 29, 29, and 7 respectively. The actual number of families following the three types of behaviour is thus not far from expectation.

4. White flowered plants, whether from F_2 or F_3 families, invariably bred true.

5. Purples from F_3 families which exhibited the 3 : 1 type of ratio behaved according to expectation. Of 18 families grown, 12 segregated into purple and white in the 3–1 ratio, while 6 bred true.

6. Purples from F_3 families which produced the 9 : 7 ratio were not grown.

It is clear that the hypothesis advanced to explain the experimental results is confirmed at practically all points.

Correlation of Flower Colour with Anthocyanin Colouration of Seed and Vegetative Parts.

The purple flowered F_1 of the cross between the two white flowered forms St Vincent Bush and Vilmorin's Stringless exhibited (*a*) purple colouration of the nodes of the main axis, and to a slight extent of the veins of the leaf, (*b*) black seeds. In subsequent generations all plants with purple flowers possessed purple nodes, and *vice-versa*. One of the parents, St Vincent Bush, is devoid of anthocyanin colouration in all parts of the plant, including the seeds which are cream. The other parent, Vilmorin's Stringless, possesses an extremely minute degree of pigmentation of the stipular hairs. The presence of colour can only be detected microscopically. The seeds, however, are of a purplish brown colour, and sections of the seed coat showed that the pigmentation was partially anthocyanic.

It has been pointed out by Morgan *et al.* (1915), that a single factor difference may have not one, but many effects. On flower colour alone it is not possible to distinguish between the phenotypes **Cr**, **cR**, and **cr**, for they are all white flowered. The factor **C**, however, produces visible effects in other parts of the plant body, and the combination **Cr** may thus be easily distinguished from **cR** and **cr**. So far as we know there are no means of distinguishing **cR** and **cr**.

The factor **C** produces a purplish brown anthocyanin colouration of the seed coat, and a minute amount of pigmentation of the stipular hairs.

The F_1 of the cross **Cr** by **cR** is coloured purple in the region of the nodes along the main axis, while the seeds are black. In subsequent generations all purple flowered, i.e. all plants with both **C** and **R**, have black seeds and purple nodes. It thus appears that the combination of **C** and **R** determines a particular mode of distribution of anthocyanin pigmentation in the plant body. The factor **C** determines the production of anthocyanin in seed and in stipular hairs. The factor **R** has no effect except in presence of **C**, when it converts white flower into purple, brown seed into black, at the same time extending the pigmentation of the

vegetative parts to include the nodal regions of the stem. The cross St Vincent Bush by Vilmorin's Stringless may be represented thus:

Parents	St Vincent Bush **cR**	× Vilmorin's Stringless **Cr**
	White seed White flower Green plant body	Brown seed White flower Purple stipular hairs
F_1	**Cc Rr** Black seed Purple flower Purple nodes	

F_2	9 **CR**	3 **Cr**	3 **cR**	1 **cr**
	Black seed Purple flower Purple nodes	Brown seed White flower Purple stipular hairs	White seed White flower Green plant body	White seed White flower Green plant body

It is clear that the best classification of the plants could be effected by examining the seed colour. The F_2 of the cross **Cr** by **cR** would show in respect of seed colour 9 black, 3 brown, and 4 white. In the actual experiments all the plants could not be examined for this character, as many matured too late. Of 77 plants which were white flowered, 33 were brown seeded, and 44 white seeded. These numbers correspond exactly with expectation on the basis of a 3 : 4 ratio.

General Summary.

In a cross between two varieties of *Dolichos lablab* the inheritance of growth habit and distribution of anthocyanin pigmentation of flower, seed, and vegetative parts have been studied. The Mendelian factors which govern these characters are as follows:

I. The factor for indeterminate habit.

C. The factor for purplish brown seed coat and purple stipular hairs.

R. A factor which has no effect except in presence of **C**, when it converts white flower into purple, brown seed into black, and causes pigmentation of the nodal regions.

REFERENCES.

Bateson, W., Punnett, R. C., and Saunders, E. R. 1905. *Reports to the Evolution Committee of the Royal Society, II.*

Emerson, R. W. 1916. "A genetic study of plant height in *Phaseolus vulgaris*." *Neb. A. E. S. Research Bull.* 7.

Harland, S. C. 1919. "Inheritance of certain characters in the cowpea." *Journ. of Genetics*, Vol. viii. No. 2.

Morgan, T. H., Sturtevant, A. H., Muller, H. J., and Bridges, C. B. 1915. *The Mechanism of Mendelian Heredity.*

Saunders, E. R. 1909. Cited in *Mendel's Principles of Heredity*, W. Bateson, p. 95.

Waby, J. F. 1917. *Journ. Bd. Agric. British Guiana.*

Wheldale, M. 1907. "Inheritance of Flower Colour in *Antirrhinum majus*." *Proc. Roy. Soc.* Vol. lxxix. B.

STUDIES ON VARIATION IN THE MEALWORM, *TENEBRIO MOLITOR.*

By S. A. ARENDSEN HEIN.

I. BIOLOGICAL AND GENETICAL NOTES ON *TENEBRIO MOLITOR.*

(With 16 Text-figures and 1 Diagram.)

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In genetic investigations made on *Tenebrio molitor* the occasion presented itself for making various observations of a more biological and systematic nature. Some of these are ripe for communication. Of the genetic investigations a more detailed report will follow later on.

A. The Larva.

1. *Colour.*

When observing a population of *Tenebrio* larvae, furnished by the trade, one will in most cases be struck by the fact that the colour of these larvae is very variable. If the culture under observation embraces a large number of individuals, two markedly distinct colour-types may nearly always be isolated, viz.:

a. Larvae of a light orange red colour, which will henceforth be called the *OR* larvae (orange red).

b. Larvae in which the orange red is replaced by a dark chestnut brown, designated by the name of the *CB* larvae (chestnut brown).

If fortune favours us, a third type, as an exceptional case, may be observed, viz. larvae that are still darker (sometimes considerably darker) than the *CB* larvae, and which, on account of the jet black colour of the ventral abdominal segments of the adult beetle, will be distinguished from the two others as the *BA* larvae (black abdomen).

Besides these three types, one will meet with a large number of intermediate colours in which the orange red may be blended with the chestnut brown so as to produce many shades of reddish brown and brownish red.

The *BA* larva is very rare and is not so easy to isolate as a pure strain as the *OR* or *CB* larvae.

This third type (*BA*) will be referred to again in the discussion of the beetle. For the moment we shall confine our attention to the *OR* or *CB* larva which are easy to distinguish.

When from these isolated types some generations are bred, they prove to be (with respect to their colours) homozygous races whose cross-breeding produces, in the F_1 generation, the immediate colours referred to above.

At first I thought that the *CB* larva was identical with the larva of *T. obscurus*, and the *OR* larva with *T. molitor*.

In the extensive literature on Coleoptera the larva of *T. obscurus* is designated as dark brown in contradistinction to the larva of *T. molitor*, which is described as being red. Curtis gives of the larva of *T. obscurus* a coloured picture corresponding entirely to the *CB* larva. Besides one

sometimes finds the statement that both species of larva (*T. obscurus* and *T. molitor*) are often found together in the same culture (Everts, p. 265; Przibram).

When the beetles of our two types were bred it soon appeared that my presumption was wrong; the beetles of the *OR* and *CB* larvae are *T. molitor* beetles, which in spite of a very extensive comparative research do not differ phenotypically, or if they do differ it is only in the black colour of the adult *CB* beetle which, as a rule, is more intense (although not always). So it is highly probable that the above statements in the literature are wrong in so far as the *CB* larvae have been taken for the larvae of *T. obscurus*, without any further research; in other words, it was not suspected that the larvae of *T. molitor* may present themselves in two colour-types, the *OR* and the *CB* type which are no longer to be distinguished with certainty in the adult beetles.

2. *Larval Anomalies.*

In almost any somewhat extensive culture the following three anomalies may be observed.

a. Larvae with one or two pairs of rudimentary wings.

b. Larvae of which two or more segments are partially fused.

c. Larvae with an increased or decreased number of spines at the distal extremity of the last segment.

a. Rudimentary Wings.

On the 3rd and 4th segments[1] the rudimentary wings appear as flat horizontal outgrowths about half the length of the segment (see Fig. 1).

Fig. 1.

This anomaly was only met with in larvae of an abnormally long larval stage.

[1] For the sake of simplicity the head is called the 1st segment, which expression, speaking morphologically, is not correct, though it simplifies the description and orientation.

b. Segmental Anomalies.

The number of segments of which the larval body is built up amounts to twelve, besides the head. Of these segments two or more may be partially fused in various manners. Rather than describe each case apart I refer to Fig. 2 (Nos. 1—25), where these fusions are represented in diagram.

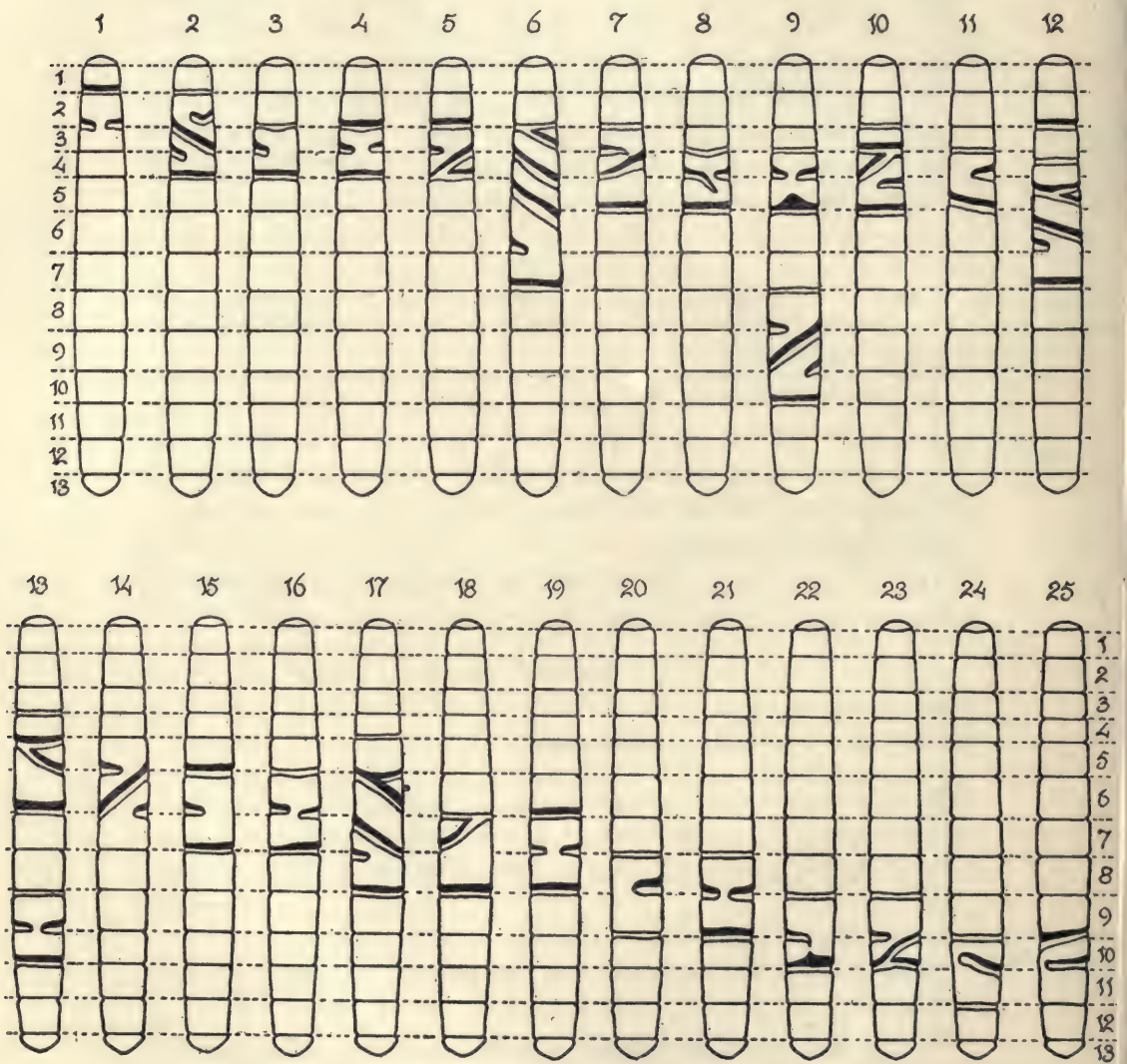


Fig. 2.

Entirely or partially fused may be

the	2nd + 3rd	segments,	Fig. 2,	Nos. 1, 2
„	3rd + 4th	„	„	2, 3, 4, 5, 6, 7
„	4th + 5th	„	„	6, 8, 9, 10, 11
„	5th + 6th	„	„	6, 12, 13, 14
„	6th + 7th	„	„	6, 12, 14, 15, 16, 17
„	7th + 8th	„	„	17, 18, 19
„	8th + 9th	„	„	9, 20, 21
„	9th + 10th	„	„	9, 13, 22
„	10th + 11th	„	„	24, 25.

From the 2nd, 3rd and 4th segments spring the larval legs. When these segments are partially fused it may occur that the two fused halves carry only one leg, as is the case in No. 3 and No. 7.

In No. 3 the right hand half of the 3rd segment was fused with the corresponding half of the 4th segment; the right leg of the 3rd segment had not been developed. The same phenomenon presents itself in No. 7, where the right leg of the 3rd segment was wanting.

Remarkable is also the instance given in No. 6. Here several segments are partially fused in such a manner that the connecting ligament between the segments form a spiral line.

When examining a population of larvae, supplied by the trade, I found, in any 300 or 400 larvae, one with an abnormal segment. The 10,000 individuals examined yielded 35 larvae with abnormal segments. Of these 18 beetles could be bred, which were paired.

Successively three generations were bred from them. The F_1 generation produced only one abnormal larva. In the F_2 generation among the 884 larvae there were seven with abnormal segments. The F_3 generation of these seven larvae was, however, normal again. Afterwards the experiment was repeated once more on a larger scale with larvae from my own cultures. Among 38,800 investigated larvae there were 62 with abnormal segments.

A segmental anomaly maintains itself in the pupa, of which some instances are figured in Fig. 3, 1—4, and once more in the beetle, where it causes strange deformities. One of these monstrosities is shown in Fig. 4. The prothorax consists here of two halves lying symmetrically to the median line and forming two equilateral triangles, whose tops point to each other. Not always are these halves so equal and similar as is shown in the figure. Sometimes one of the halves is more strongly developed than the other, or is deformed by dints and deep

grooves. This strange anomaly also did not reappear even in the F_2 generation. So the result has been negative as regards the heredity of segment-anomalies.

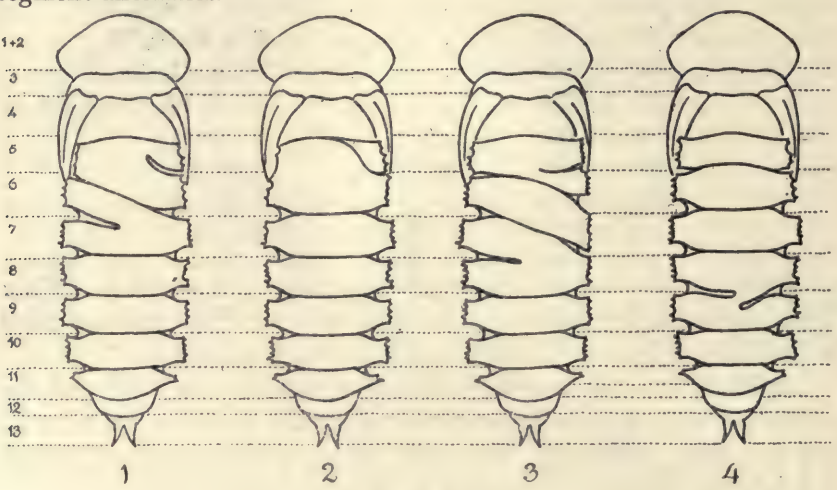


Fig. 3.

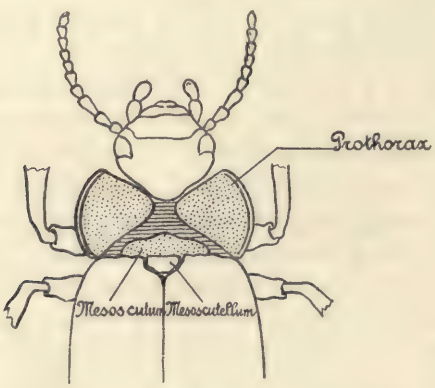


Fig. 4.

c. Variation of the Number of Spines on the Last Segment.

The larva of *T. molitor* carries at the end of the last (13th) segment two black-brown chitinous spines, which distally slightly diverge. To the right and left (laterally and a little proximally to the base of these spines) may be found still on the same segment, two dark points, which, when observed under a more powerful lens, also appear to be spines but only in a rudimentary state.

These rudimentary accessory spines are sometimes as an abnormality strongly developed.

The following cases, differing from the normal state, occurred:

1°. Four spines, of which one to the left, and three (joined in a group) to the right of the median-line (Fig. 5, No. 1) (1 individual).

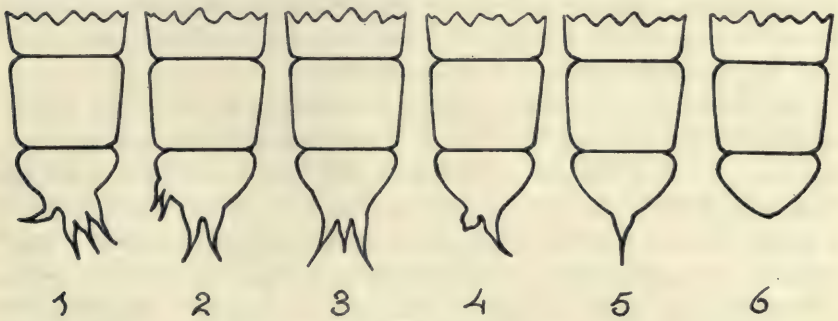


Fig. 5.

2°. Four spines, of which two in the normal place, the other two are found on one side, somewhat below the boundary between the last segment, and the last but one.

These last two spines are abnormally developed accessory spines, of which mention was made above (Fig. 5, No. 2) (1 individual).

3°. Three spines, the middle one of which lies in the median-line, the other two symmetrically to the left and right of it (Fig. 5, No. 3) (1 individual).

4°. Two spines, of which one normal and one rudimentary (Fig. 5, No. 4) (4 individuals).

5°. One well developed spine, lying in the median-line (Fig. 5, No. 5) (7 individuals).

6°. Total *absence* of spines (Fig. 5, No. 6) (2 individuals).

All those variations, which were isolated from a number of 10,000 larvae, are maintained again in the pupae of these larvae, as is shown by Nos. 1—5 of Fig. 6.

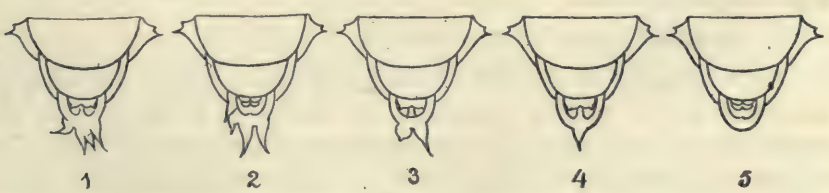


Fig. 6.

Of those six conditions the fifth case, viz. the one spine lying exactly in the median-line, was the most important for the object of these investigations, because the one-spine character of larva and pupa is a specific character of *T. picipes*, which is distinguished from all other *Tenebrio* species in this particular aspect. The relatively larger number of individuals, too, seemed to be an indication that the one-spine character was not an anomaly of equal value with the five other cases, but that I had here to deal with *T. picipes.*

Of these one-spine larvae (with corresponding pupae) three generations were bred of which, however, all the larvae displayed the normal two spines. Also the subsequent crossings of normal ♂ × one-spine ♀ and the reciprocal cross did not give any result. Later on, when examining again a great number of larvae in search of single spined individuals, I found cases in which the normal two spines were lying close beside almost in contact with each other (Fig. 7, Nos. 1 and 2). Larvae of this kind gave

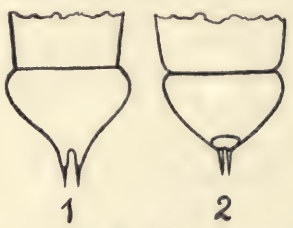


Fig. 7.

pupae with only one spine, lying exactly in the median-line. When such spines were examined under a powerful lens, it could be seen that the spine was split; sometimes only at the top, sometimes as far down as past the middle. Thus the so-called unpaired median spine appeared, in several cases, to be paired.

On the other hand, however, cases came under observation, where the double nature of the single spine, even by magnifying it still stronger (object-glass A, ocular 2), could *not* be ascertained, either in the larva or in the pupa obtained from it. This individual, too, gave in cross-breeding a negative result.

3. *Duration of the Larval Period.*

If the influence of the kind of food is left out of account, the developmental stages as growth, pupation, the emerging of the beetles, the copulation, the depositing and development of the eggs are in a high degree dependent on the temperature. This strong sensibility to influences of temperature enables us to shorten the developmental stages

from egg to beetle, i.e. it is possible to get the beetles at one's disposal at any time of the year. This gives the great advantage, that the work, which often accumulates suddenly in experiments with cross-breeding, can be divided over a longer period.

The temperature of the incubators varied from 22½°—27½°, averaging 25° C. In this temperature, and in favourable feeding-conditions, the duration of the larval period varies from 5 to 10 or 12 months. By far the greater number of the larvae pupated within 11 months. But every culture produces also larvae which pupate much later, and whose larval period lasts 14—18 months. Even with the children of one and the same mother the larval period may differ widely.

How far hereditary factors, too, play a part here, will be treated in another paper. For the present we may as well say that the investigation is impeded in a high degree by the great influence of outward circumstances. I have not been able to confirm from my experiments the information here and there to be found in literature (Krizenecky, p. 54) that insufficient feeding at a certain moment would be favourable to pupation or, what is the same, that the larval stage would be shortened[1].

4. *Number of Moults.*

It is a rather astonishing fact that in the literature[2] the statement can always be found that the larva moults four times, and that after the fourth moult the pupa appears. This assertion has evidently never been tested because a simple experiment would immediately have shown its fallacy. In our experiments undertaken for that purpose the number of moults was never less than 10 and never more than 19. In a series from 8 larvae originating from the same mother and born on the same day the number of moults were 13—12—14—11—13—13—14—13 with a larval period of 187 days in 7 larvae and 209 days in one larva.

B. The Pupa.

1. *Differences of Sex.*

It was of no small importance for the purpose of the experiments when it was found that the difference of sex in the pupae (once recognized) is so easy to ascertain that any inexperienced assistant can soon be made familiar with it.

[1] I have discussed this topic along with others in a paper "Technical experiences in the breeding of *Tenebrio molitor*," published in the proceedings of the *R. Ac. Sci. Amsterdam*, 1920.

[2] Brehm, p. 128. Frisch, Vol. III. p. 1. Saling, pp. 2—8. Sturm, pp. 21, 22.

When the pupae are examined on the ventral side the last segment shows the aspect in the ♂ and the ♀ reproduced in Fig. 8. The following differences can be stated.

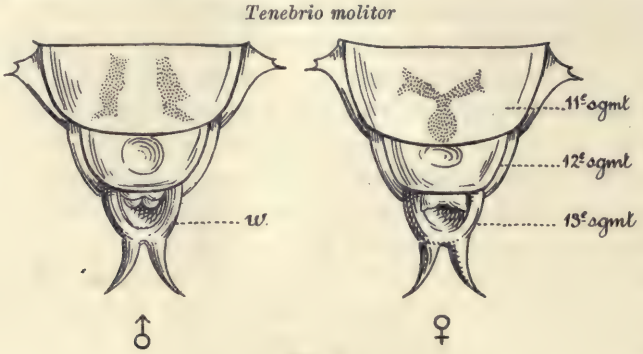


Fig. 8.

a. On the last or 13th segment arise the two diverging spines. This segment is deeply cup-shaped, partially surrounded by a semi-annular thickened edge (*w*) which encloses the segment laterally and distally. The proximal edge of the segment is not visible as it is covered by the distal edge of the last segment but one (12th). In the ♂ some four little knobs, like pin's heads, lie against and under this distal edge of the 12th segment in the proximal part of the cup-shaped hollow (or sometimes filling up this hollow for the greater part). The two proximal knobs form one whole, meet each other in a slightly curved line, and cover partly the two distal ones, which are more independent and separated from each other.

In the female only one pair of knobs is found, fused with each other, and these project farther in the hollow of the segment than the first pair in the male. Moreover these knobs are not round but drawn to a point.

A further acquaintance with the literature showed that Saling, too, had already observed these sex-differences between the ♂ and ♀ pupae, but without figuring them. There are still 3 other differences, which Saling probably did not see, as he does not mention them. They are less conspicuous, but if one knows them, they are distinct enough to be applied as tests in case of doubt. These differences are the following:

b. The distal edge of the last segment but one has in the ♀ the form of the vertical section of an U iron, the corners of which are rounded, whereas this form in the ♂ approaches a semi-circle.

c. This 12th segment has in the ♂ a cup-shaped dint; this dint is in the ♀ either absent or much less deep, and indistinct.

d. The tissue of the segments, dimly seen through the chitinous skin, has a dull creamy-yellow colour in which in the 11th segment of the ♂, to the left and right of the median-line, is seen a peculiarly curved figure, the concavity of which is directed laterally. Both figures lie symmetrically to each other, and have a semi-opaque grey colour, like an oil-stain on white paper.

In the ♀ these paired figures meet in the median-line of the 11th segment, and continue unpaired distally; the whole figure has the shape of a two-pronged fork, with a short handle, thickened in the form of a ball while the opening of the fork is directed proximally. In the ♂ this fork-figure is lacking.

By means of these four characteristics, of which the first is the most distinct and preferable to all others, the investigator will always be able to determine the sex with absolute certainty.

The above-mentioned sex-differences make it not only possible to isolate male and female beetles before their emergence, but they also provide a means of answering the following questions among others:

(α) Is the number of males and females alike or does either of the sexes predominate?

(β) Is there any difference in the average length of the female and male beetle, and, if so, what is the proportion?

2. *The Ratio of the Sexes.*

In the course of 3 months 3000 pupae were examined as to sex, and after that, measured. These pupae belonged to one group, being a mixed population brought together from different places in Holland. They were obtained and measured in the following months:

The 1st thousand from the 2nd of April—3rd May 1915.
The 2nd thousand from the 4th of May—the 4th of June.
The 3rd thousand from the 5th of June—the 28th of June.

The sex ratio of this group was as follows:

Month	Total of measured pupae	Number		Number of females to 1000 males
		Male	Female	
April ...	1000	557	443	795·33
May ...	1000	448	552	1232·16
June ...	1000	492	508	1032·52
Totals and Ratio	3000	1497	1503	1004 ♀ to 1000 ♂

Males and females are represented here in nearly equal numbers, viz. 1004 ♀ to 1000 ♂.

In the beginning of the month of April the first pupae appeared. The males were at first in the majority, and that in such a manner that 3 male individuals were obtained to 1 female. Gradually the number of females increased, yet not so much but that in the end of that month this ratio was still 125 ♂—100 ♀. In the month of May the females appeared in a greater number, so that by the end of May the ratio was the reverse of that in the end of April, namely 100 ♂—123 ♀. Only in the month of June ♂ and ♀ appeared in about equal numbers, namely 100 ♂—103 ♀. The result over the whole gathering-period was as is mentioned above viz. 1000 ♂—1004 ♀.

This original predominance of the males did not, strange to say, reappear in my own cultures, in subsequent years. In these as a rule the appearance of males and females was generally numerically equal in the various periods of the gathering. Perhaps the environmental conditions, which were in my cultures, of course, much more equable than in trade populations, play a part in this phenomenon.

The ratio of the sexes was verified once more in a great number of divers cultures of the generations 1918, 1919 and 1920. In all, 13,754 pupae were examined, which were represented by 6866 males and 6888 females, i.e. 1000 ♂—1003 ♀.

3. *The Difference in Length between the Sexes.*

Larvae and beetles are difficult to measure in a living condition. Through fidgeting and uneasiness, and other causes, they give rise to very unreliable figures in measuring. The pupae are very uneasy when measured immediately after their emergence from the larvae. They turn round, stretch themselves, contract or make all kinds of contortions; in short they try to resist the operation.

Also the softness of their body renders it generally impossible to determine their length exactly. If, however, the pupae are left to themselves in the incubator for 48 hours, then all difficulties are at an end. The body of the pupa has become a firm tractable object by the hardening of the chitinous skin. Its movements are much less violent, they quiet down sooner, so that we find no longer any difficulties.

The mode by which the pupae, whose shape is as a rule strongly crescentic, were measured will be explained more clearly in another paper, and the results of the investigation as to length will be given in connection with hereditary and environmental factors.

We revert again to our two groups, the measures of which are as follows:

First group	Males: Number of measured pupae	Males: Average length in mm.	Females: Number of measured pupae	Females: Average length in mm.	Sexual ratio
First thousand (April) ...	557	13·01	443	12·94	1·005
Second thousand (May) ...	448	14·12	552	13·68	1·032
Third thousand (June) ...	492	15·60	508	15·38	1·014
Total	1497	14·24	1503	14·00	1·017
Second group					
Fourth thousand (June) ...	442	15·47	558	15·28	1·012
Total of first and second group	1939	14·48	2061	14·37	1·007

Though in each group of 1000 pupae the females are somewhat smaller than the males, this difference is still so small (the average sex-relation, the ♀ taken as unity, is only 1·007) that it may safely be disregarded, when in questions of heredity as regards length the average length of a brood or a strain has to be ascertained.

The results of these measurements are also interesting in another respect. When the average is calculated for each thousand (i.e. of ♂ and ♀ together) of the 1st group, the figures of which are immediately mutually comparable, the result is as follows:

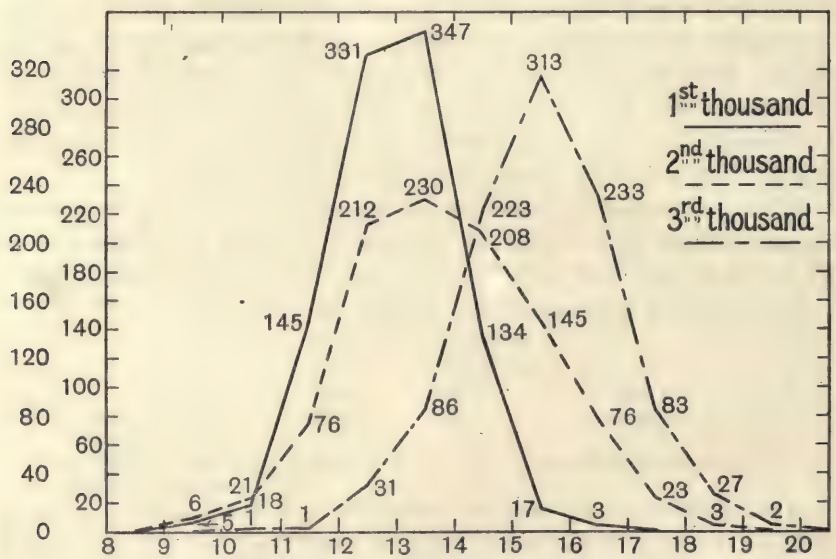
	Number of measured pupae	Average length in mm.	
First thousand (April) ...	1000	12·98	
Second thousand (May) ...	1000	13·88	0·9 mm. longer than the first thousand
Third thousand (June) ...	1000	15·49	1·6 mm. longer than the second thousand

It appears therefore that the average length increases every month; the 2nd thousand measured in May is on an average ± 1 mm. longer than the 1st thousand of April; the 3rd thousand of June again ± 1·5 mm. longer than the 2nd thousand of May.

When each of these three variation-series is represented in a curve, one observes that the curve, as a whole, shifts to the right every month. This phenomenon repeated itself in the collection of the following year in every culture of a series in which pupae of different lengths were kept for some generations (measure-series). In many of these single cultures it could be observed that the curve was gradually getting two peaks. It produced the impression of two strains differing genotypically (not only in their precocity and tardy maturity, but also in their lengths) and separating themselves from the mixture. This is, however, probably not the case, and it would seem to me that external conditions alone are the principal, if not the only governing factor of this phenomenon.

It is not the design of this paper to enter into particulars about this problem, the less so, because later on I shall refer to it again with more complete documentary data. Here I only draw attention to the fact that, independent of the season, the smallest individuals of a certain culture appear in a decided preponderance in the beginning, the larger beetles in a later period of that same culture.

The following table and curves (in which also the values characteristic of the three variation-series are stated) give a graphic illustration of the fact mentioned.



	Number of individuals measured n	Average length $M \pm m$ Mean ± probable error $m=\frac{\sigma}{\sqrt{n}}$	Standard deviation $\sigma=r\sqrt{\frac{\Sigma pD^2}{n}}$	Coefficient of variability $v=\frac{100\sigma}{M}$	Skewness S $S=\frac{\Sigma pD^3}{n}:\sigma^3$	Excess E $E=\left(\frac{\Sigma pD^4}{n}:\sigma^4\right)-3$
1st thousand	1000	12·98 ± 0·033	1·0553	8·13	− 0·064	+ 0·237
2nd ,,	1000	13·88 ± 0·056	1·6031	11·55	+ 0·120	− 0·263
3rd ,,	1000	15·49 ± 0·041	1·3141	8·48	− 0·06	+ 0·100

When determining the coefficient of variability for the males and females separately, I found it for the males to be higher than for the females, and this was so in each of the 3 series of 1000 pupae. The exact figures are:

		Males	Females
1st thousand	...	8·57	7·52
2nd ,,	...	12·07	10·86
3rd ,,	...	8·95	7·92
	Average	$v=12·52$	11·50

C. The Beetle.

1. *Secondary Sexual Characters.*

In the literature the following sexual characters are mentioned:

for the ♂ a stronger thickening of the femur,
a stronger curvature of the tibia,
a comb-like row of hairs along the inner edge of the tibia.

When the sexes are compared as to these points it is found that the number of cases in which the first and second differences *cannot* be stated is much greater than the cases in which that greater curvature of the tibia or thickening of the femur can be observed. It is quite certain that the difference in curvature and thickening is so slight and inconstant that it is unserviceable for distinguishing the sexes.

In my researches it was of great practical importance to ascertain with absolute certainty the sex of the living beetles. This gave rise to a comparative examination of all the organs and parts of organs in both sexes. The result was satisfactory and only the following three characteristic differences, being the most striking, need be mentioned[1].

a. The Hairiness of the Tibia.

Over and above the presence of some accumbent yellow hairs in both sexes, the tibia of the ♂ carries, along the median edge, a row of erect hairs which was never observed in the ♀.

Especially in larger beetles this row of hairs is particularly well developed, and has the form of a comb.

This row of hairs may however be not very conspicuous and, not seldom, is wanting altogether. When the hairs are present this character, so easy to ascertain quickly is of good service in the separation of the sexes.

b. The Shape of the last Ventral Abdominal Segment.

Referring to Fig. 9 the following facts may be observed. The distal edge of the last or 5th abdominal segment has in the ♂ the form of a part of a circle; in the ♀ this segment is somewhat sharper and more cone-shaped. Though this difference in the shape of the 5th segment is clearly discernible (after some practice), yet another characteristic

[1] I followed in this research the indications given by Professor Moll as applied to botanical objects, called by him the method of "Pen-portraits," i.e. a scrutiny and description of the structures in their minutest details as far as is observable with the power of the lens chosen for this purpose. (See J. W. Moll, "De beschrijving der simplicia in de Nederlandsche Pharmacopae." *Pharmaceutisch weekblad*, 1916, Nos. 42, 43.)

difference, namely the character of the connective tissue between the 4th and 5th segment, is *in many cases* still more striking.

In the ♂ as well as in the ♀ the separation between the 1st and 2nd, and also between the 2nd and 3rd segments, shows only *one* line, being the distal edge of the most anterior segment, which after the fashion of a tile covers the front edge of the following segment. The separation-lines between the 3rd and 4th and between the 4th and 5th segments are in the ♂, as well as in the ♀, no longer a single line, but a double one. Both lines form together the arc of a circle with the chord of that arc; the arc is the distal edge of the preceding, the chord the proximal edge of the following segment. The space between chord and arc (which in the ♂ is strikingly larger because the distal edge of the 4th segment is much more strongly concave) is spanned by a light coloured membrane (connective skin between the two segments)

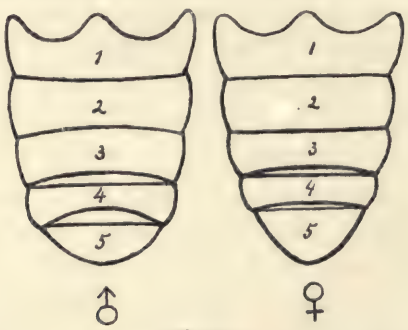


Fig. 9.

which as a rule contrasts strongly with the dark coloured surroundings, and is consequently, by its larger surface, immediately observed in the ♂.

By the greater concavity of the distal edge of the 4th segment in the ♂, this segment has, in longitudinal direction, become narrower than in the ♀, where this narrowing is very slight or absent.

c. Shape of the Ventral Valve, which covers the Exterior Sexual Organs.

Penis and ovipositor are covered dorsally and ventrally by a crescent shaped plate, or valve (Fig. 10). In the ♂ the ventral valve is in the median-line divided into two lobes by a deep incision. In the ♀ this incision is wanting, and the distal edge of this ventral valve forms an uninterrupted curved line. Both valves (the dorsal and the ventral) are invisible in beetles that have attained their adult colorations, as

they are covered completely dorsally by the elytra, ventrally by the last abdominal segment.

When the beetle is turned on its back and the abdomen is pressed with a stiff pencil, then emerges in the ♂ the valve with its deep incision, in the ♀ the ovipositor (partially still telescoped) so that the sex can be determined immediately, by this feature, as well as by the two palpi at the distal end of the ovipositor.

The deep incision in the valve in the ♂ serves apparently to give passage to the penis, lying dorsal to it, when this organ during the copulation bends ventrally and in a forward direction[1].

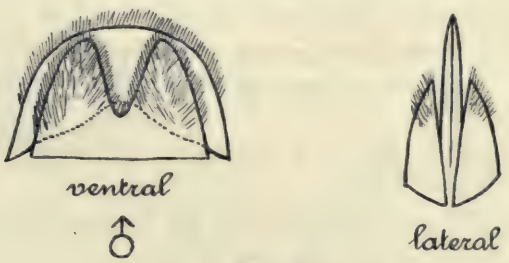


Fig. 10.

In connection with this we can understand why the membranes between the 3rd and 4th and between the 4th and 5th segments are more conspicuous in the ♂ than in the ♀, as has been mentioned above. For these ample membranes render a hinge-like movement possible in the segments concerned, a movement which occurs when in the ♂ those segments are bent ventrally during the coition.

Of the above-mentioned characters this last is infallible.

In the thousands of beetles which have been examined as to the hairs of the tibia, there occurred not seldom cases (as has already been mentioned) where in the ♂ a row of erect hairs was wanting, whereas this absence is normal in the ♀ without exception up to the present.

But the sex-differences mentioned above, will give almost absolute certainty.

2. *Duration and Quantity of the Egg-Production.*

The eggs are deposited not in batches, but one by one, isolated. They have a milk-white colour, are oblong-oval in shape, 1·75 mm.

[1] After this paper had been sent to the editors of the *Journal of Genetics*, I became acquainted with the study of Kerschner dealing with the male copulation organ of *T. molitor* published in *Zoologische Jahrbücher*, Bd. xxxvi. in which the author has given a sketch of the valve mentioned above, and of the male and female pupae.

long, 0·5 to ·75 mm. broad. Through the simple way in which the deposited eggs could be gathered and counted the following facts could be ascertained:

a. When both sexes of beetles, after development of their adult coloration, are brought together, the first eggs may in most cases be gathered within 8 days. By way of exception this time is reduced to 1 or 2 days, or may be extended to a fortnight. If after a fortnight no eggs are deposited it is almost certain that no fertilized eggs will be obtained. This sterility of the beetles is sometimes very annoying, especially when one wishes to breed rare variations. Fortunately it is exceptional, though not so very rare.

b. The ability to produce a larger or smaller quantity of eggs is a highly variable character, the average figure of which could be only determined from a great number of experiments. From one female 30 eggs as a minimum, 359 as a maximum were obtained. A normal harvest gives 100—150 eggs.

c. No regularity or periodicity in the egg-production could be ascertained either in the quantity laid in a day, or in a definite period of days. Sometimes eggs can be gathered daily during a week, sometimes a period of 2—14 days passes between two succeeding gatherings. Even if the numbers of eggs obtained during periods of 5, 10 or 15 days are compared, the figures show nothing that would point to any regularity.

d. The duration of the egg-production is also very irregular and varies between 50 and 130 days. Normally the gathering will last 2 months before egg-production ceases.

3. *Duration of Life of the Sexes.*

The male does not live so long as the female. When only one pair is brought together the ♂ is usually killed by violence and eaten by the ♀, often in such a manner that only a few remains of the chitinous organs are recovered. When the ♀ has filled her vesicula seminalis with sperm, and she no longer needs the ♂ for the fertilization of her eggs, she seems no longer to care for his caresses, and he is killed. She continues then for quite a long time to produce fertilized eggs. From a series of 32 experiments (undertaken with the view of ascertaining the duration of life of the sexes) it appeared, that on an average the male lives 60 days, and the female 111 days. So the ♀ survives the ♂ by about 51 days.

The shortest duration of life of the ♂ was 39 days
„ longest „ „ ♂ „ 113 „
„ shortest „ „ ♀ „ 89 „
„ longest „ „ ♀ „ 132 „

After the death of the ♂, the ♀ goes on regularly depositing fertilized eggs. As an extreme case I observed that a female deposited 2 fertilized eggs, 63 days after the death of the ♂. As a widow she had produced as many as 95 eggs in those 63 days. So it is clear, that once the ♀ has copulated, she is not fit for cross-breeding experiments with another ♂, supposing she copulated more than once.

For this, and other reasons, it was of great importance for these researches that I should be able to determine the sex of the beetles immediately after the throwing off their pupal skin.

4. *The Copulation.*

In these experiments there occurred circumstances in which it was desirable to know whether a ♂ copulates with more than one ♀, in other words whether a ♂ brought together simultaneously with a number of females fertilizes more than one of them. And also conversely: the question whether the ♀ copulates more than once was of some interest.

For the first case the course of the experiment was simple. In each of a series of five pots one ♂ and one ♀ were brought together. As soon as eggs appeared, they were gathered, and the ♀ replaced by another virgin ♀; then a following egg harvest was awaited, and the second replaced by a third ♀; and so on. The eggs gathered of each copulation were kept, till they were hatched to ascertain the fact that they had been fertilized. The results of this investigation are given in the table on page 246.

Of the five male beetles No. 2 died untimely. Of the four others No. 1 copulated with 16 females before he was killed by the 17th female. Male No. 3 copulated with 11 females; No. 4 and No. 5 with 10 females. Three of the males were eaten (Nos. 1, 3 and No. 5); whether the two others died a natural death could not be made out; no mutilation or traces of it were observed.

An investigation into the number of copulations of the female is much more elaborate and takes some years, because in general no indications of repeated copulation can be obtained until some succeeding generations have been kept.

She had to be crossed successively with males of different hereditary characters, and the new characters at our disposal were all reces-

sive, so that they did not appear before F_2. This experiment is not yet closed; as yet we can only say that a female can copulate more than once. Further information about it will be given later on.

In connection with the investigation as regards the heredity of certain anomalies a third question had to be answered with absolute certainty. It depended upon this answer whether the work would be greatly simplified or considerably increased. It concerned the following matter. The gathered pupae of each larval-culture had their own

Number 1

Date when the female was paired with the male	Number of pairings	Harvest of eggs: Date	Harvest of eggs: Number
13 Oct. '18	1st	21 Oct. '18	12
21 ,,	2nd	27 ,,	9
27 ,,	3rd	7 Nov.	28
7 Nov.	4th	11 ,,	9
11 ,,	5th	17 ,,	5
17 ,,	6th	27 ,,	19
27 ,,	7th	6 Dec.	19
6 Dec.	8th	10 ,,	20
10 ,,	9th	16 ,,	14
16 ,,	10th	20 ,,	19
20 ,,	11th	29 ,,	18
29 ,,	12th	5 Jan. '19	14
5 Jan. '19	13th	13 ,,	4
13 ,,	14th	19 ,,	8
19 ,,	15th	27 ,,	20
27 ,,	16th	2 Feb.	4
2 Feb.		7 ,,	♂ dead mutilated

Duration of life of the ♂ = 117 days	16	Total of eggs from 16 females	222

Number 2

Date when the female was paired with the male	Number of pairings	Harvest of eggs: Date	Harvest of eggs: Number
13 Oct. '18	1st	27 Oct.	6
27 ,,	2nd	3 Nov.	7
3 Nov.	3rd	11 ,,	4
11 ,,	4th	23 Nov.	♂ dead not mutilated

Duration of life of the ♂ = 41 days	3	Total of eggs from 3 females	17

Number 3

Date when the female was paired with the male	Number of pairings	Harvest of eggs: Date	Harvest of eggs: Number
13 Oct. '18	1st	25 Oct.	7
25 ,,	2nd	31 ,,	24
31 ,,	3rd	7 Nov.	13
7 Nov.	4th	11 ,,	13
11 ,,	5th	15 ,,	19
15 ,,	6th	23 ,,	5
23 ,,	7th	28 ,,	4
28 ,,	8th	6 Dec.	22
6 Dec.	9th	13 ,,	21
13 ,,	10th	20 ,,	44
20 ,,	11th	27 ,,	24
27 ,,	12th	13 Jan.	♂ dead mutilated

Duration of life of the ♂ = 77 days	11	Total of eggs from 11 females	196

Number 4

Date when the female was paired with the male	Number of pairings	Harvest of eggs: Date	Harvest of eggs: Number
13 Oct. '18	1st	21 Oct.	17
21 ,,	2nd	31 ,,	32
31 ,,	3rd	7 Nov.	20
7 Nov.	4th	9 ,,	11
9 ,,	5th	15 ,,	12
15 ,,	6th	19 ,,	7
19 ,,	7th	27 ,,	5
27 ,,	8th	6 Dec.	12
6 Dec.	9th	10 ,,	9
10 ,,	10th	29 ,,	11
		29 Dec.	♂ dead not mutilated

Duration of life of the ♂ = 58 days	10	Total of eggs from 10 females	136

Number 5

Date when the female was paired with the male	Number of pairings	Harvest of eggs	
		Date	Number
13 Oct. '18	1st	21 Oct.	11
21 ,,	2nd	3 Nov.	6
3 Nov.	3rd	9 ,,	13
9 ,,	4th	15 ,,	27
15 ,,	5th	20 ,,	12
20 ,,	6th	27 ,,	9
27 ,,	7th	6 Dec.	7
6 Dec.	8th	8 ,,	5
8 ,,	9th	13 ,,	20
13 ,,	10th	20 ,,	35
20 ,,		29 Dec.	♂ dead mutilated
Duration of life of the ♂ =68 days	10	Total of eggs from 10 females	145

pupae-dish, in which the emergence of the beetles was awaited, and as soon as these came out they were examined. Sometimes in F_1, but as a rule in the F_2 generation the beetles which did *not* possess a given character had to be removed. Those which showed it had to be kept for further breeding. I had to be quite sure, however, that the beetles had not already copulated before, otherwise a separation would not have been of any use. The pupae-dishes were daily inspected for the presence of beetles; so the beetles examined were at most 24—30 hours old.

The question to be answered was: how much time will elapse between the beetle being hatched and its copulation. If copulation should take place within ± 36 hours, it would be necessary to arrange a male and a female pupae-dish for every culture. An answer in this sense would cause not only a doubling of the number of the dishes, and of incubator space, but also a not inconsiderable increase of work (separation of the sexes). The problem was solved by the following experiment. Some beetles from the moment they were hatched were kept two by two (♂ and ♀) in small pots in the incubator for 72 hours. After this time all the males were removed, and the females kept for one month for the gathering of eggs, if any.

From this series of 10 pairs, eggs were obtained from only two females; one producing one egg, the other eight eggs. The nine eggs were apparently sterile as they produced no larvae, and after some time all were shrivelled. They were probably virginal eggs, which some females will produce when prevented from mating. The result of this experiment leads to the conclusion that no copulation takes place within thirty hours after the hatching.

5. *Sterile Eggs.*

We had already observed a few times that isolated, virgin females occasionally deposit eggs, which fact suggested the possibility of parthenogenesis, of which but few instances are recorded in Coleoptera. In a series of ten dishes we put a number of females (mostly 25 in each dish), and kept these for 25 days. 248 beetles in total were isolated, from

Number of beetles..	25	15	25	35	25	25	25	25	25	23
					Eggs obtained from					
Date	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
14 April 1916	1	—	—	—	—	—	—	—	—	—
16	1	—	—	—	—	—	—	—	—	—
17	2	—	—	—	—	—	—	—	—	—
19	7	2	—	—	—	—	—	—	—	—
22	2	—	—	—	—	—	—	—	—	—
23	3	—	—	—	—	—	—	—	—	—
24	3	1	—	—	—	—	—	—	—	—
25	5	1	—	—	—	—	—	—	—	—
26	14	3	—	—	—	—	—	—	—	—
27	2	—	5	2	—	—	—	—	—	—
28	4	3	3	1	—	—	—	—	—	—
29	1	—	5	1	—	—	—	—	—	—
30	3	—	1	1	—	—	—	—	—	—
1 May	2	—	2	1	1	—	—	—	—	—
2	1	1	—	—	—	—	—	—	—	—
3	1	1	1	1	—	—	—	—	—	—
4	4	1	—	—	1	—	—	—	—	—
5	—	2	2	—	—	1	3	—	—	—
6	—	—	4	—	1	—	—	—	—	—
8	—	—	8	2	—	—	2	—	—	—
10	—	—	1	1	—	—	—	—	—	—
11	—	—	5	5	1	3	1	—	—	—
12	—	—	1	3	1	4	—	1	—	—
13	—	—	8	2	—	2	—	2	—	—
15	—	—	2	1	—	2	—	4	—	—
17	—	—	—	—	—	2	8	3	—	—
18	—	—	—	—	5	—	—	—	2	—
19	—	—	—	—	—	2	2	1	1	—
20	—	—	—	—	—	1	—	1	—	—
22	—	—	—	—	—	4	4	—	—	—
24	—	—	—	—	—	—	3	—	—	—
27	—	—	—	—	—	—	—	—	1	—
3 June	—	—	—	—	—	—	—	—	16	—
8	—	—	—	—	—	—	—	—	—	4
9	—	—	—	—	—	—	—	—	—	1
10	—	—	—	—	—	—	—	—	—	2
26	—	—	—	—	—	—	—	—	—	12
1 July	—	—	—	—	—	—	—	—	—	9
Total Eggs	56	15	48	21	10	21	23	12	20	28

which 254 eggs were obtained. The table on page 248 supplies a survey of the number of eggs deposited by each group of beetles, together with the dates on which they were laid. All the eggs gathered were put in the incubator; none of them were developed; after some time all were shrivelled.

It cannot be concluded from this experiment whether oviposition (without previous mating) is proper to many beetles or to a few individuals only. The 56 eggs produced by group No. 1 may originate from one as well as from all the 24 beetles. But then the experiment had not been made in view of an answer to this problem. I wished only to know whether non-fertilized eggs can be hatched parthenogenetically. We may safely conclude, from the result obtained, that, when left to themselves, without the application of artificial means, they do not develop as a rule. I shall refer to the problem of artificial parthenogenesis in due course when the experiments made in this line have come to an end.

6. *Development of the Adult Coloration.*

Not all the organs of the beetle complete their coloration simultaneously. In which organs the pigment shows itself first may best be observed in the pupa. The mandibles and the eyes differentiate first from the surrounding parts and are already very dark when the antennae and the legs display only the beginning of a brown colouring. Shortly before the casting of the pupal moult the condition of the pupa is as follows:

Eyes	black.
Mandibles	
Head	reddish brown.
Antennae	
Tibia and tarsus of the 6 legs	
Distal edge of the last abdominal segment	
Prothorax	light orange yellow.

The rest of the pupal body is still uncoloured, not to mention those parts that are removed with the pupal skin and no more present or conspicuous in the beetle, such as the spines on the last segment and the spines on the lateral edges of the abdominal segments. In the beetle just emerging from the pupal skin the light orange-red prothorax and the deep black eyes contrast strongly with the white of the elytra. An examination of the organs in this stage will show the following facts:

Eyes	black.
Mandibles	
Head	light to chestnut brown. The episternum is considerably darker than the epimerum.
Antennae	
Mouth parts	
Mesopleura	
Metapleura	
Trochanter, Tibia, Tarsus of the 6 legs	
5th abdominal segment	
Prothorax	light orange red.
3rd and 4th abdominal segment	white with a yellow brown spot on right and left.
Coxae	white, with brown spot above the cotyle.
Femur	white, excepting the fore and hind edges, which are light brown.
Neck	entirely white.
Elytra	
Prothorax (ventrally) ...	
Mesosternum	
Metasternum	
1st and 2nd abdominal segment	
Scutellum	

From this initial stage till the adult coloration of the beetle some time elapses that may be shortened considerably by a rise in temperature. This influence on the speed of coloration may be gathered from the table on page 251.

Here we have to revert again to the *BA* larva, mentioned on p. 228. Beforehand the fact should be borne in mind that no constant difference can be observed between the *OR* and the *CB* beetles, either in the course of the development of their coloration or in the individuals that have completed their colouring. In general the black colour of the *CB* beetles is deeper than that of the *OR* beetles, whose hue is often (by no means always) more a chocolate brown.

The beetles of the *BA* larvae are distinguished from the beetles of

the *OR* and the *CB* larvae in that they do not display the red brown colour in any stage of the colouring process.

Number of hours since the birth of the beetle	Group A of 4 beetles at 26° C.	Group B of 4 beetles at 13° C.	
7½	Evenly light brown	Grey	
24	Evenly brown	Not yet light brown	
50	1 beetle blackish brown. 3 beetles dark brown	All light chestnut brown	
76	1 beetle black. 3 beetles nearly black	Still light chestnut brown	The difference in colour between the two groups is strikingly great
99	Same as after 76 hours	Light chestnut brown with a dark shade here and there	
312 (13 days)	Same as after 76 hours	No adult colour on any of the beetles yet: two were still chestnut brown	

As was stated before the *BA* beetle is very rare. If it occasionally appears at all as a single individual freshly emerged amidst the mixed population of *CB* and *OR* beetles, it immediately attracts attention by the striking contrast of black and white. All that was stated above to be light brown and dark brown in the newly emerged *CB* and *OR* beetles, is in the *BA* beetle black or a shade of black, which appears at first diffusely in the white body, giving when coloration is complete to the whole body with its appendages that coal black tint by which the *BA* beetle is marked off from the *OR* and the *CB* beetle. In the latter the legs, antennae and abdominal segments, also after their final colouring, preserve a red brown, or, at any rate, a much lighter hue than the rest of the body. In the *BA* beetle all these organs are uniformly coal black.

In addition to this difference in colour of the organs just mentioned, it is characteristic of the *BA* beetle that the black colour appears in the very first stage of the pigmentation process, i.e. in the pupa, and later in the freshly emerged beetle at the *same time* and on exactly the *same parts* of the organs as the red-brown in the *OR* or *CB* beetle.

In the latter the black (in the adult coloured beetle) is less intense. It mostly shows still a tinge of the initial red-brown, and displays itself

much later, but not before the beetle has passed a stage in which its whole body is of a uniform red-brown.

About crossing experiments between $OR \times CB$, $OR \times BA$, and $CB \times BA$ we shall report in a subsequent paper.

Besides the above deviations in the colour of the *T. molitor* beetle there may still be recorded one about which I am yet uncertain whether, though in some cases due to a change in conditions, it may not be caused by a genotypical "Anlage" in other cases. It occurs not rarely that the *OR* and *CB* beetles assume not a final black but a final brown hue. This brown, here the last stage of the adult colouring, appears in different shades. Often the light colour is confined to the prothorax, sometimes to the wings alone, and in a minority of cases the whole beetle has a light hue.

In order to be able to state the amount of difference between the normally coloured beetle and the light coloured individuals one should observe them both in a glass dish on a coal black background; beetles then showing a clear difference of colour, may scarcely exhibit this when examined on a white ground. Among 10,661 beetles examined, which were kept for eight days in the incubator during the coloration process, there were 165 whose colour on a white ground (i.e. under unfavourable conditions for comparison) displayed a conspicuous light brown tint, *i.e.* about 15 *per mill.*

An F_1 generation of these beetles was kept. The same selective process was applied to it in behalf of an F_2, and later again of an F_3 and F_4 generation. In this way I hoped to isolate a strain which, as to the final stage of the coloration, differed genotypically from the more general type whose adult colour is nearly black. These selective experiments are not yet finished, and I will refrain here from any anticipatory judgment about the possible result.

As far as my experience goes it is beyond any doubt that an abnormal course of the colouring process may be brought about either by disturbances in the development of the beetle during the pupal stage, or by other outward circumstances the nature of which I am not yet able to define.

7. *Hereditary and Non-Hereditary Anomalies.*

The cardinal point of these investigations lay in the search for deviations from the normal type in order to study them with a view to their behaviour in heredity. In this I have been but slightly influenced by

a priori considerations about the genotypic value of these deviations; in other words I did not prejudge the possibility whether some deviation should be regarded as a pathological disturbance in the development or as a reaction of a changed complex of gens. Looking back I do not regret having taken this point of view, in spite of the extension of the labour it entailed. For not seldom were the results contrary to expectation, often even when I had to deal with variations of taxonomic value.

The following list gives a survey of the most important deviations which were observed and tried as to their hereditary character. When nothing is mentioned about the latter it means that either no positive result has been obtained, or that the experiments are not yet closed. The hereditable deviations will be treated with full data in subsequent papers.

a. The Eyes.

No. 1 *a*. The normal colour of the eyes is intensely black. In very rare cases they are cream-white, here spoken of as *yellow*, and when observed for the first time, this condition was mostly found in ♂ beetles. On one occasion it was noticed in ♀ individuals in a population bought in the trade. The yellow eye-colour is sex-limited in its descent, following the rule known for colour-blindness in man, and exemplified by the many recessive sex-linked characters investigated by Morgan in *Drosophila*.

Black-eyed ♀ × yellow-eyed ♂ gives all F_1 black-eyed, ♀s and ♂s alike. In F_2 from this cross, yellow-eyed ♂s reappear.

Yellow-eyed ♀ × black-eyed ♂ gives the ♀s black-eyed and the ♂s yellow-eyed; and in F_2 from this cross all four forms are produced. The numbers will be given when sufficient data have been obtained, and, together with certain aberrations which have been met with, will be discussed in a subsequent paper.

No. 1 *b*. In other cases the eye colour may be red. This anomaly appears not to be sex-limited.

No. 2. One of the eyes may be rudimentary without the other organs on that side of the head being influenced by this.

No. 3. One of the eyes may be entirely wanting. In the few cases that occurred the right or left eye was absent. This fact was associated with a partial development of the right or left half of the head; the right antenna or that part of the clypeus overarching the basis of the antennae was lacking also.

b. The Antennae.

The variations in these organs appear as a reduction, an increase, or a deformity of the segments. A decrease of the number of segments is not quite so rare as an increase, which was met with only twice. The normal number of segments is eleven.

No. 4. Cases were noted in which this number was reduced to 10, 9, 8, 7, 6, 5, 4 and 3 segments, or

No. 5. The whole antenna was wanting, and for that matter, the clypeus was imperfectly developed, though both eyes were present.

Most of these reductions are only unilateral; the other antenna is often normal. The reduced antenna not infrequently bears indications of the process by which that reduction was brought about, viz. by fusion of two or more segments.

Such a fusion may affect various segments, excepting the scapus and the pedicellus in which no fusion was ever observed.

No. 6. In those cases where the antennae consisted of nine or ten segments it was often noticed that all nine or ten segments, of one or both antennae, had a normal length and shape without any indications of the reduction having been caused by a fusion of segments.

No. 7. In some of these cases it was accompanied by a reduction of the tarsal segments on all six legs. In one culture of about 100 beetles this anomaly was observed nine times, which seems to be based on a hereditary factor, but about which no particulars can be given yet.

No. 8. An increase of the number of the segments is very rare. It was found only twice, and not in a complete form. The case consisted in the 9th segments in both antennae being abnormally enlarged, and showing an obvious constriction, indicating that this segment is built up of two joints.

No. 9. Deformities, when the number of segments is normal, manifest themselves in an enlargement or diminution of one of the segments, in excrescences or spine-like processes, or in a fan-like arrangement of the segments which are constricted then on one side only.

c. The Prothorax.

Many of the variations in the prothorax were of a quantitative nature. They concerned the degree of development of some one or other morphological character, slight differences, which, on account of the smallness of the objects, were difficult, if not impossible, to express quantitatively. The personal experience of the observer and his intimacy with the material must decide his judgment of what is called weak or strong.

Gradual transitions from one extreme to another, the usual fluctuating modifications, render the classification difficult. Still it appears from the experiments that some of these variations conform to a definite type, so that they can hardly be taken as extreme variants of one series if in the progeny of such a type these extremes return in very large numbers. It is not the place here to enter upon any details of this sort of quantitative variation. In this paper I shall confine myself only to the description of rare deviations of a qualitative nature which (although they modify the shape of the prothorax) do not affect the bilaterally symmetrical condition of this structure.

Fig. 11 c^3 shows the shape of the prothorax as it is found generally. It is seen that the distal edge displays two small notches right and left.

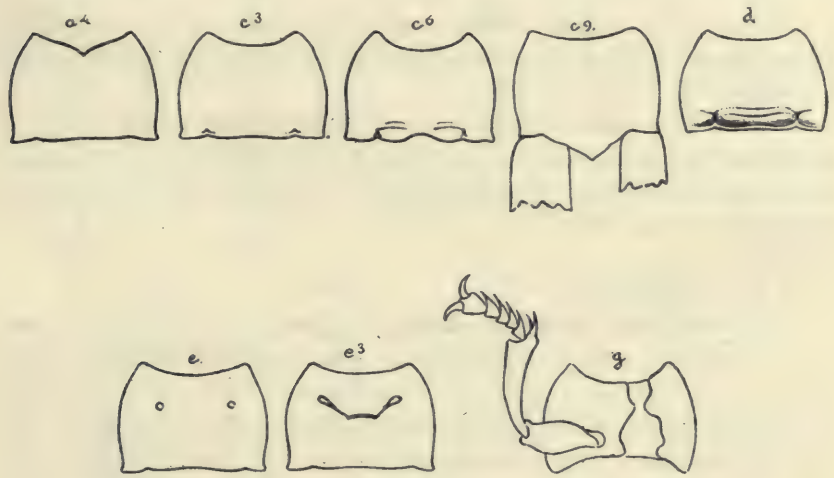


Fig. 11.

No. 10. Sometimes this distal edge is anteriorly scalloped in the median line as shown in Fig. 11 c^6.

No. 11. It may also occur that the reverse presents itself, and the posterior edge, in the median line, has grown into an isosceles triangle, the vertex of which is directed distally. Fig. 11 c^9.

No. 12. Something like this may also occur in the anterior edge, which, in normal cases, is slightly concave, but it also may appear as a crooked line, the two arms of which, like the two sides of a triangle, converge medially.

No. 13. Occasionally an abnormal cupola-shaped arching both of the dorsal and of the ventral plane of the prothorax was found.

No. 14. That the prothorax may be built up of two symmetrical disconnected halves (Fig. 4, p. 232) has been mentioned above already.

No. 15. In consequence of a segmental anomaly in the larva, it was also once remarked that the left half of the prothorax was imperfectly developed with the whole leg, including the coxa, lacking.

This absence of the leg was already noticed in the larva (Fig. 11 *g*). Also the deviations No. 11 and No. 14 were found in beetles that were distinguished by anomalies of the segments in the larval state. I shall pass over the numerous asymmetrical deformities of the anterior, posterior and lateral edges that bear the aspect of accidental disturbances in the development.

d. The Transverse Rib.

Directly rostral to the distal prothoracic edge and bordering it, in *T. obscurus* is found an elevation (*Querwulst, bourrelet transversal*) which I will name the transverse rib.

No. 16. This transverse rib is rarely, in *T. molitor*, found in such a prominent form as is indicated in Fig. 11 *d*. Between this development and its complete absence a gradual series of more abundant transitional forms may be met with.

e. Grooves and Dints.

Besides the usual fine punctulation, the dorsal surface of the prothorax is rarely smooth. Generally there are found grooves, crater-like pits (Fig. 11 *e*) or dints of various forms and extent. As a rule these pits or dints are paired and lie symmetrically to the median line; seldom are they unpaired, as a single dint in the right or left half, but they never occur in the median line.

No. 17. A very curious form of cavity is pictured in Fig. 11 e^3, where a deep transverse groove right and left continues into another groove which, running anteriorly and sideways, ends in a broadened dint.

f. Scutellum.

The variations in the scutellum are few in number and not conspicuous. Such a perfect semi-circular scutellum as *T. picipes* possesses I have never observed in *T. molitor*, though cases were noticed approaching it very nearly.

No. 18. Only twice could the absence of the scutellum be established, once in the same beetle that produced the prothoracic anomaly mentioned in No. 15.

g. The Elytra.

The variations in these organs presented themselves mainly:

1°. *As a shortening or a lengthening of one or of both elytra.*

The elytra look perfectly normal for that matter, and do not suggest that the anomaly has arisen from a developmental disturbance, as many other reductions do which are accompanied with deformities of the wings.

No. 19. Quite an exceptional case is the shortening of one elytron only; the abdomen on that side is then not wholly covered (Fig. 12 *a*).

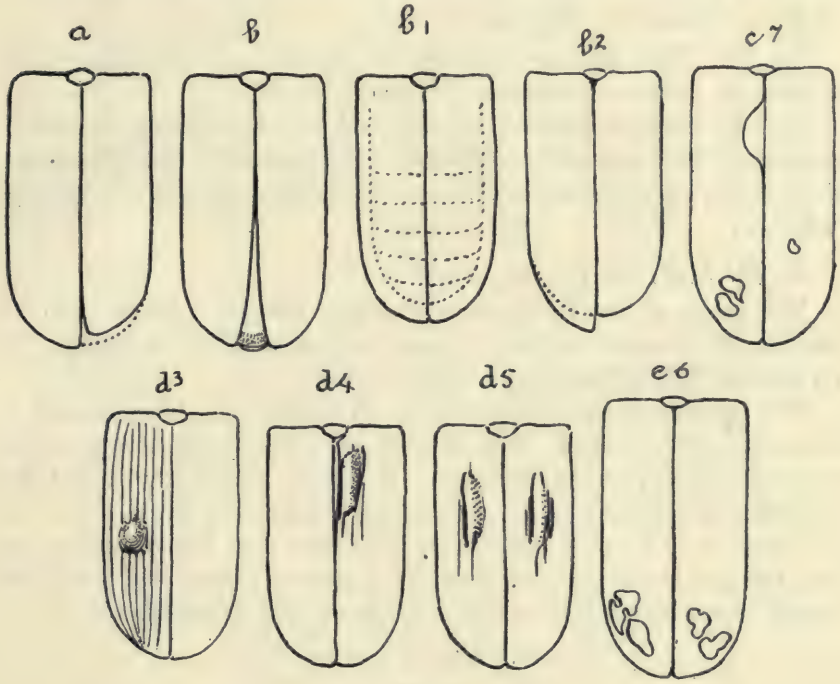


Fig. 12.

No. 20. More than once both wing-covers were found shortened.

No. 21. The case of the elongation of both elytra shown in Fig. 12 b^1 is rare.

No. 22. So also is that of one elytron being prolonged as in Fig. 12 b^2. (The dotted line in the figures indicates the outline of the abdomen.)

2°. *As a divergence of the median elytral edges.*

Normally the median elytral edges touch each other in their whole length.

No. 23. It may occur, however, that even from the posterior 3rd part of the elytra the edges diverge, so that a part of the abdomen is visible (Fig. 12 *b*), or the divergence is only small and confined to the distal part of the elytron.

No. 24. Sometimes this phenomenon is accompanied with a reduction of the elytra.

3°. *As perforations.*

No. 25. The wings display holes of various shapes, number, situation and extent, either on one wing-cover or on both (Fig. 12, c^7 and e^6).

4°. *As blister-like swellings.*

No. 26. They may occur on any part of either or both wing-covers, in different shapes and extents (Fig. 12 d^3; d^4; d^5).

The variations mentioned under 3° and 4° are evidently the consequences of developmental disturbances. The reduction and divergence of the elytra are frequently normal specific characteristics in other groups of beetles.

h. The Legs; the Tarsus.

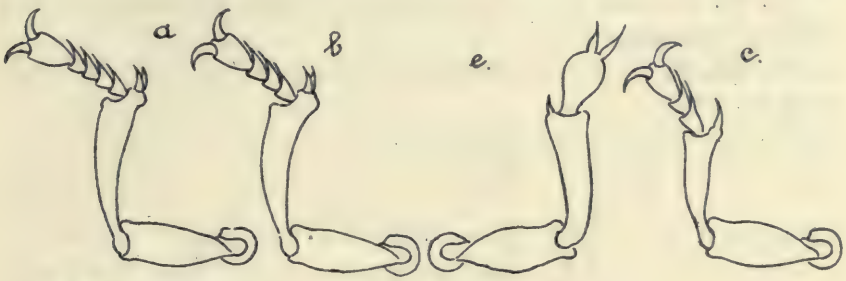
The group of the Heteromera, to which *Tenebrio* belongs, owes its name to the unequal number of tarsal segments, which is five for the 1st and 2nd pair of legs, four for the 3rd pair.

The number of tarsal segments in *T. molitor* may be increased or decreased. This decrease may be observed (though not always simultaneously) in all three pairs of legs. It may go to the extent that the tarsus finally consists of only one segment bearing the claws.

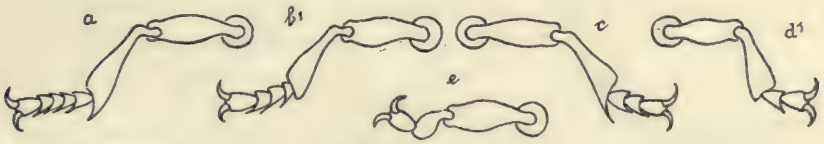
Often there is not a trace of any indication that the reduction has been brought about by a fusion of the segments. These are then of a normal length and shape, as the under-mentioned figures show.

No. 27.	Fig. 13 *b*	1st leg.
No. 28.	„ *c*	
No. 29.	„ *e*	
No. 30.	Fig. 14 b^1	2nd leg.
No. 31.	„ *c*	
No. 32.	„ d^1	
No. 33.	„ *e*	
No. 34.	Fig. 15 *c*	3rd leg.
No. 35.	„ *d*	
No. 36.	„ *e*	

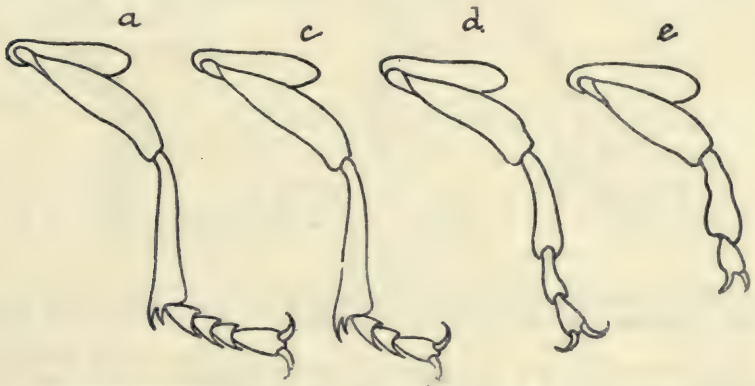
On the other hand there are cases where in the reduced segment (both by its length and the said constrictions) it is clearly observable that the reduction arose from a fusion of the segments, even though



Normal condition. Fig. 13. 1st leg.



Normal condition. Fig. 14. 2nd leg.



Normal condition. Fig. 15. 3rd leg.

the number of those constrictions is often less than the number of segments the leg normally possesses. (Fig. 16 *d* and d^1.)

The numerical tarsal reduction may appear only in one leg or in both legs of the pair.

Conditions were observed where the tarsi of all six legs were reduced, and this condition appeared, after isolation, to be hereditary, so that strains could be reared in which all beetles showed the same anomaly in each successive generation.

No. 38. An increase of the number of tarsal elements may occur, either *only* in the third pair of legs, either in one leg or in both legs; the tarsus then consists of five segments;

No. 39. Or only in the second pair of legs; the tarsus is then built up of six segments;

No. 40. Or in the second and third pair of legs (the first pair being normal);

No. 41. Or in all legs. Fig. 16 a^3.

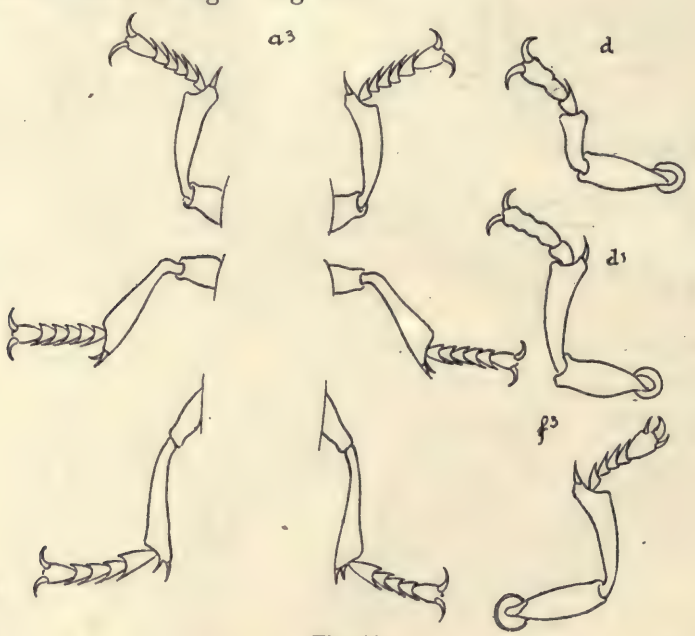


Fig. 16.

a^3, six legs of beetle having a supernumerary joint in each tarsus. d and d^1, tarsi imperfectly divided. f^3, reduplication of apical joint.

The increase is only one joint above the normal, so that the above-mentioned cases showed the following number of tarsal segments.

	First pair of legs	Second pair of legs	Third pair of legs
No. 38	5	5	5
No. 39	5	6	4
No. 40	5	6	5
No. 41	6	6	5

In consequence of the positive results obtained with respect to the heredity of a *reduced* number of tarsal segments I have paid much attention to the above-mentioned cases of an *increased* number of joints,

and rather specially to the condition when the tarsus of all three pairs of legs consisted of five segments. (No. 38.)

From 35,247 beetles tested as to their tarsi, 60 individuals were found whose third pair of legs showed five segments.

Three generations were bred of these, without any result. If the deviation did reappear at all, the frequency of it was not greater than in any population taken at random.

Nos. 42, 43. Of the two claws on the last tarsal segment, one or both may be lacking. The single claw then lies in the axial line of the tarsus.

No. 44. Equally rare is the case pictured in Fig. 16 f^3, where the tarsus bears three claws. [This is evidently a form of reduplication of the terminal joint.] Normally the two claws diverge and are curved outwardly. Sometimes they are straight and parallel to each other and to the axial line of the tarsus.

i. Femur and Tibia.

No. 45. The decrease of the number of tarsal segments may or may not be accompanied with a reduction of the tibia alone, or of the femur and tibia together.

No. 46. Femur and tibia may, however, be reduced with a normally built tarsus.

No. 47. The two spurs on the distal extremity of the medial edge of the tibia may be lacking, which is often associated with a reduction of the number of tarsal segments of the leg.

No. 48. Not quite so rare is the absence of one leg or both legs of a pair, in which case, however, coxa and trochanter are still extant.

Only in intenser disturbances it may occur (as was mentioned already in No. 15, Fig. 11 *g*) that even of these two elements of the leg no rudiments were left.

No. 49. A very singular anomaly which was found only once, consisted in a fusing of the two coxae of the first pair of legs into one, on which both legs (otherwise normally built) were implanted. Also the two articular cavities were fused into one single cotyle, lying exactly in the median line.

Numerous other anomalies may occur in the legs, upon which we shall not enter now. They consist in the appearance of excrescences, accessory spines in places where spines do not normally occur, abnormal thickenings, reduplication of the tarsus, whether or not provided with the normal number of tarsal segments, etc.

A general survey of the principal results obtained with these investigations, leads to the following:

Summary.

I. *The Larva.*

1. In nearly every population of larvae of *Tenebrio molitor* bought in the trade, two colour varieties are found, viz. the *CB* larva (chestnut brown) and the *OR* larva (orange red).

2. As a rare exception a third type may be met with, viz. the *BA* larva (black abdomen), which is distinguished from the two above-mentioned ones, in that its colour is still darker than the *CB* larva.

3. The most conspicuous larval anomalies exhibit themselves:

as a development of the rudimentary wings;
as a partial fusion of the segments;
as a variation in the number and position of the spines occurring on the last segment.

4. The variations mentioned in 1 and 2 are hereditary, those in 3 are not.

5. The duration of the larval stage, under otherwise favourable nutrition-conditions, is in a high degree dependent on temperature. By raising or lowering the temperature the duration can be considerably shortened or lengthened.

6. In spite of high temperature and favourable nutrition-conditions, the larval stage may vary between $\pm$ 5 and $\pm$ 18 months. It is possible that hereditary factors may play a part in this.

The number of moults varies greatly.

II. *The Pupa.*

7. The male and the female pupae are easy to recognise by a difference of morphological characters.

8. The sex ratio, determined on a large number of individuals, may be taken as equal.

9. The difference in dimension between the male and the female pupa is small; the sexual ratio amounts only to 1·007 if the female is taken as unity. The coefficient of variability is for the males higher than for the females (♂ $v = 12{\cdot}52$; ♀ $v = 11{\cdot}50$).

III. *The Beetle.*

10. The size of the beetle varies between 10·5 and 23·5 millimeters.

11. The male and the female exhibit secondary sexual characters which facilitate the separation of the sexes.

12. The number of eggs produced by one female is very variable. As a maximum 359 eggs were obtained from one individual.

13. Also the duration of the egg-production varies very much. In the normal condition it lasts two months.

14. The duration of life of the female exceeds that of the male. In a series of experiments the ♀ was found to survive the ♂ by about 51 days.

15. The male mates with more than one female. It was established as a fact that one male had copulated with 16 females.

16. The mating of the beetles does not take place within 48 hours after the pupal moult has been cast.

17. The number of sterile eggs produced by a virgin ♀ is small, presumably there may be only a few. Most females produce no eggs without previous mating.

18. Between the *CB* and *OR* beetles no morphological differences can be observed; the individual of the *CB* beetle, that has attained its adult colour, is as a rule darker than that of the *OR* beetle.

19. The *BA* beetle is a melanistic form. It differs from the *CB* and the *OR* beetle in:

(*a*) That the beetle during the final coloration does not pass through a stage of red brown, as the *CB* and *OR* beetles do.

(*b*) That the abdomen, antennae and legs are coal black, whereas these organs are red brown in the *CB* and *OR* beetles.

20. It has been established that, besides some quantitative variations, the yellow and red colour of the eyes and some forms of reduction of the number of tarsal and antennal segments, are based on hereditary factors. The yellow eye-colour has a sex-limited descent, but the red appears to be not sex-limited. Strains can be reared which, with respect to these abnormalities, breed true.

Utrecht, *February* 1920.

LITERATURE.

1. Brehm. *Tierleben*, Bd. ix. Insekten. 3e Auflage, 1892.
2. Curtis, John. *British Entomology*, 1823, Vol. vii.
3. Everts, Ed. "Coleoptera Neerlandica." *De schildvleugelige insekten van Nederland*, 1903.
4. Frenzel. "Ueber Bau und Thätigkeit des Verdauungskanals der Larve des *Tenebrio molitor*." *Berliner Entomolog. Zeitschr.* Bd. xxvi. 1882.
5. Frisch, J. L. *Beschreibung von allerlei Insekten in Teutschland*, 1720.
6. Krizenecky, Jar. "Ueber die beschleunigende Einwirkung des Hungers auf die Metamorphose." *Biolog. Centralblatt*, Bd. xxxiv. 1914.
7. Przibram, Hans. "Die biologische Versuchsanstalt in Wien (Zweck—Einrichtung—Tätigkeit, etc.)." *Zeitschr. f. Biologische Technik und Methodik*, 1910.
8. Saling, Th. "Zur Kenntnis der Entwickelung der Keimdrüsen von *Tenebrio molitor*." *Inaugural Dissertation, Marburg*, 1906.
9. Sturm, Jacob. "Deutschlands Fauna." *Abbildungen u. Beschreibungen v. Insekten—Käfer.*

VOLUME X DECEMBER, 1920 No. 4

NOTE ON AN ALTERNATING PREPONDERANCE OF MALES AND FEMALES IN FISH, AND ITS POSSIBLE SIGNIFICANCE.

BY JULIAN S. HUXLEY.

IN a paper published some years ago Mr E. G. Boulenger (1912) mentioned that the sex-ratio of some "Millions Fish" (*Girardinus poeciloides*), bred by him in the Zoological Gardens, was approximately 3 ♀ : 1 ♂.

In private conversation and in letters, Mr Boulenger has recently informed me that this state of affairs continued for nearly a year, the preponderance of females being observed in nearly every brood. During the winter, however, the sex-ratio changed, and there was a preponderance of males, but this preponderance was not so great as that of the females had been. He has unfortunately no record of the actual figures, but is sure that the preponderance was less; he places the new sex-ratio at about 2 ♀ : 3 ♂. This second period lasted only a short time (not more than six weeks), and was succeeded by a third in which approximately equal numbers of males and females were produced. The sex-ratio continued thus (1 ♀ : 1 ♂) for the remainder of the time during which the fish were bred at the Gardens—a period of several years.

Most of the fish had been some time at the Gardens before Mr Boulenger came there, having been received from Barbados about 1909. The sex-ratio during that time was not recorded. A few were received from Kew Gardens during his own experiments. All, however, belonged to the same sub-species. The sex-ratio in the wild state is not known, but is presumed to be 1 : 1.

The experiments were not carried out with a view to results of genetic interest, but to furnish a supply of Girardinus for various tropical stations, in order to devour mosquito larvae. About 150 adults were kept. The number of young in a brood is from 5—25. A new

brood is produced about every 14 days at 70° F.; but one impregnation will suffice for two, three, or more broods. It is thus very difficult to say how many generations were involved in the whole period of altered sex-ratio. The period of male preponderance would seem to be that of a single generation (impregnation). During this period the preponderance of males was noted in approximately six out of eight broods.

We can thus distinguish (*a*) a period of 9—10 months with a preponderance of females (3 ♀ : 1 ♂); (*b*) a period of a few weeks, with a lesser preponderance of males (1 ♀ : 1·5 or 2 ♂); (*c*) a subsequent period of several years with the presumably normal sex-ratio (1 ♀ : 1 ♂).

I wish here to point out that these results are capable of interpretation on a hypothesis which has considerable theoretical interest, and, further, is capable of verification.

It is common knowledge that in many animals of different groups sex-determination is performed by the chromosomes, one sex possessing one sex-chromosome, the other two. The sex with one sex-chromosome (heterogametic) may be either male or female.

In the first case, we have the composition ♂ *XY*, ♀ *XX*; while all the eggs are alike, two classes of spermatozoa are produced.

In the second case, the composition is symbolized as ♂ *ZZ*, ♀ *ZW*; there are two classes of eggs, but only one of spermatozoa.

It might at first sight be supposed that this determination of sex by the chromosomes was fixed and unmodifiable; but there exists considerable evidence to show that the sex-ratio may in certain cases be modified by external factors. The most startling work is perhaps that of R. Hertwig (1907) and Kuschakewitsch (1910), who showed in frogs that, by using stale eggs, the percentage of males was greatly increased, in some cultures no females appearing at all. (It may be remarked that this result appears definitely *not* to have been due to differential mortality.)

The existence of sex-chromosomes has not actually been demonstrated for frogs, although this has been done for Necturus, where the male has only one *X*, and there are therefore two kinds of spermatozoa (King, '12). If we assume, however, that the frog is like the bulk of other animals which have been investigated in possessing sex-chromosomes, and further that it resembles its relative Necturus in being of the ♀ *XX*, ♂ *XY* type, we must suppose that the 50 % of eggs which were fertilized by *X* sperm and, being *XX* in composition, should normally have become females, were under the conditions of the experiment transformed into males.

This could have been accomplished in one of two ways. Either one of the *X*-chromosomes was transformed into a *Y*—a hypothesis so improbable as not to be worth further consideration; or else the normal effect of two *X*-chromosomes was overridden by the effect of the delayed fertilization upon the eggs' metabolism.

If this latter hypothesis is adopted, then we must assume that 50 °/₀ of one of these "all-male" cultures are normal, of constitution *XY*, but that the other 50 °/₀ are what may be called "masculinized females," or, better, to adopt Shull's useful term ('14), *somatic males*, of constitution *XX*.

All of this latter class of males, if bred to normal females under normal conditions, should give only female offspring, since all spermatozoa as well as all eggs in such a cross will contain an *X*-chromosome. The remaining males mated in a similar way will give the normal 1 : 1 sex-ratio, so that the effect of mating normal females to a number of males selected at random from an all-male culture will be to give a preponderance of females in the next generation in the proportion 3 ♀ : 1 ♂ (a less preponderance than the previous preponderance of males). Both males and females, however, of this generation will be of normal constitution, so that their offspring would show a normal 1 : 1 sex-ratio.

Similar effects of late fertilization on sex-ratio are given by Pearl and Parshley ('13) for cattle.

Other important evidence in favour of the possibility of external factors overriding sex-chromosome constitution is botanical, and is given by Shull ('14) in his paper on *Lychnis dioica* (see especially p. 285 et seqq.).

In the first place, his experiments have led him to the conclusion that hermaphroditism in *Lychnis dioica* may be either (*a*) *genetic*, apparently due to the presence of a definite gene, and therefore heritable, or (*b*) *somatic*, due to some cause outside the germ-plasm causing a shift of a genetically unisexual individual to a hermaphrodite condition. These somatic hermaphrodites in the great majority of cases he found to behave as males, and he concludes that they are genetically males in which chromosome constitution has been overridden by external causes.

One particular hermaphrodite plant, however, when used as pollen parent in three separate crosses, gave in each case a large progeny consisting entirely of female plants. In no other experiment has Shull ever obtained even a moderate family composed only of females. His explanation is that the plant in question, though also a somatic hermaphrodite, was not a modified male but a modified female, of

constitution *XX*. In support of this conclusion based on genetical evidence, is the work of Strasburger ('00 and '10) and Doncaster ('12), who found that female plants of *L. dioica* when attacked by the anther-smut (*Ustilago violacea*) were transformed towards hermaphroditism (which is thus purely somatic in the sense adopted by Shull).

Morgan ('19, p. 188) believes that Hertwig's and Kuschakewitsch's results may be due to "late-fertilized" eggs being in reality not fertilized at all, but partheno- or arrheno-genetic. There is as yet, however, no direct evidence in favour of this view, while the case of Lychnis shows that sex-chromosome constitution may be overridden, at least so far as to give hermaphroditism.

Steinach ('12 and '13) has conclusively shown that the secondary sexual characters of male (presumably *XY*) rats can be overridden by the extirpation of the testis and implantation of an ovary; and Lillie (17') has shown that in female (presumably *XX*) embryos of cattle twinned with males, the development of the gonad itself is altered, and it and all the internal accessory sexual organs are caused to become more male than female, by means of the circulation in the blood of substances emanating from the male twin; i.e. the primary sex-characters can be modified in the zygote, and therefore apart from chromosomes. In Crustacea, Smith ('06) has given the reverse proof; the males of Inachus, upon recovery from parasitic castration, are switched over towards femaleness, ova being actually formed in their gonads.

There remains a further set of cases which are possibly capable of another interpretation. Goldschmidt ('16) and Harrison ('19) with moths and Riddle ('16) with pigeons have shown that inter-generic, -specific or -varietal crosses give a large excess, often up to 100 °/₀, of males (in these groups, the homogametic sex). In addition, Riddle has shown that a large excess of females may be produced by various depressing agents. The results are certainly not due to differential mortality. In the pigeons Strong ('12) has, however, proved that all the animals must have the chromosome-constitution normal to their sex. This he accomplished by breeding experiments involving a sex-linked character. This is further established by the data given by Riddle himself. The only hypothesis for the pigeons would therefore seem to be that the stimulating effect of foreign sperm causes the *W*-chromosome to be thrown out in the polar body, while depressing agents cause it to remain in the egg (it should be noted that at fertilization the first maturation spindle is in its metaphase). This cannot be the operating cause in the frog, if that animal is of the *XX*, *XY* type, for both egg

and polar body would then contain one *X*-chromosome. This hypothesis of differential maturation is of importance, as it involves the subordination of the chromosome-mechanism to other agencies.

As moths are of the *ZW*, *ZZ* type, differential maturation is a theoretical possibility. Since the first draft of this paper was written, however, I have not only had access to Goldschmidt's latest published statement of his results ('19), but the same author has most kindly sent me the proofs of a forthcoming paper ('20) which supersedes all his earlier work. I will not do more here than to say that the sex-ratios there obtained give further very strong evidence in favour of the possibility we have already indicated for Kuschakewitsch's frogs—viz. that zygotes with the chromosome-constitution of one sex may be transformed into functional individuals of the other sex. Not only that, but such animals, both *ZW* males and *ZZ* females, have been bred from, and, though Goldschmidt himself realizes the need for further data, all the results can be interpreted on this hypothesis, while it is difficult to account for them on any other. In other words, it appears that in these moths, abnormal sex-ratios are first produced by overriding chromosome constitution, and that sex-ratios abnormal *in the opposite direction* are produced in the next generation, exactly as in Boulenger's fish. Harrison's results ('19) on moths of the sub-family Bistoninae are in many ways similar, but are complicated by being for the most part got from species- or genus-crosses. They too are strong evidence for the possibility of individuals of the chromosome-constitution of one sex being transformed into functional individuals of the other sex, although this has not been tested out by controlled breeding experiments with such transformed individuals.

Mention should also be made of Keilin and Nuttall's intersexual lice ('19). Here again we have a graded series from "hermaphrodites" (intersexes) which are preponderatingly of one sex through those with equal development of male and female characters, to those preponderatingly of the other sex. Whether, however, this is due to overriding of chromosome-constitution cannot be said until the fuller genetic and cytological analysis which is now in progress has been made.

Let us now return to our fish. If we suppose that some external factor upset the normal sex-ratio during the first period, we have a provisional explanation of the facts. This factor must have worked in the opposite direction from late fertilization in frogs, converting animals of male constitution into somatic females. Further, in this case not all the males were thus converted. This is, however, paralleled in

many of Hertwig's experiments, where a shorter delay in fertilization gave a less preponderance of males.

The sex-chromosome formula for fish has not been definitely established, either cytologically or genetically. There is some evidence, however, that they are of the type ♀ XX, ♂ XY. For instance Gerschler ('14; see pp. 77, 78, 83) made crosses between two genera of Cyprinodonts, Xiphophorus and Platypoecilius (related to Girardinus). The former has, in both sexes, a zig-zag red band along its side; in the latter this is absent. When Xiphophorus was the female parent, the resultant F_1 fish showed this stripe in both sexes; but when Platypoecilius was the mother, only the F_1 females possessed the stripe. This would follow if Xiphophorus were of constitution ♂ XY, ♀ XX, with the factor for red zig-zag in the X-chromosome. Unfortunately the latter cross was only made with one individual, and not carried beyond F_1.

It will be seen later that if our hypothesis of the modifiability of sex-chromosome constitution by external factors prove to be correct, the case of Girardinus affords confirmatory evidence in favour of the XY, XX formula.

If then we assume that this formula does hold for Girardinus, the few male fish present during the first period would be normal, of XY constitution. At the close of the first period, when the disturbing factor ceased its action, these males mated to somatic (XY) females would give $1XX$ (♀) : $2XY$ (♂) : $1YY$ (non-viable).

The sex-ratio during the first period was given as 3 ♀ : 1 ♂. If this period had only lasted one generation, two out of every three females would be normal (XX) and one a feminized XY. It can thus easily be calculated that a swing-back after this would give a sex-ratio of 5 ♀ : 6 ♂.

Mr J. B. S. Haldane, of New College, Oxford, has pointed out to me that if the modifying influence were to continue for several generations, two effects would be produced. In the first place, the preponderance of females during the period would gradually decrease, but, on the other hand, among the females the proportion of somatic females (XY females or transformed males) would increase. This would result in a greater preponderance of males in the generation after the end of the first period (swing-back generation).

An example will make this clear. We must assume that the strength of the modifying factor remains constant—i.e. that it converts the same percentage of males into somatic females in each generation. Let us assume that the factor causes a modification of 50 % of the XY individuals in each generation. This will give us our first-generation

ratio of 3♀ : 1♂. As we have already seen, if the modifying factor were now eliminated, the sex-ratio in the next generation would be 5♀ : 6♂. If on the other hand it were to continue its action, we should have the following result:

F_1 constitution: $2XX : 2XY$.

F_1 sex-ratio: $2XX♀ + 1XY♀ : 1XY♂ = 3♀ : 1♂$.

F_1 gametes: Ova, $5X : 1Y$. Spermatozoa, $1X : 1Y$.

F_2 constitution: $5XX : 6XY : 1YY$ (non-viable).

50 % of the XY's will again be converted into females, so that the F_2 sex-ratio will be $5XX♀ + 3XY♀ : 3XY♂ = 8♀ : 3♂$.

F_2 gametes: Ova, $13X : 3Y$. Spermatozoa, $1X : 1Y$.

F_3 constitution: $13XX : 16XY : 3YY$.

The swung-back sex-ratio, if the modifying factor were not to act on this generation, would thus be 13♀ : 16♂, a preponderance of males which is greater than if the modifying influence had ceased a generation earlier.

If the modifying factor were to continue its action, the sex-ratio of F_3 would be $13XX♀ + 8XY♀ : 8XY♂ = 21♀ : 16♂$. This shows a less preponderance of females than the first modified generation, but the proportion of XY females is higher ($\frac{8}{13}$ instead of $\frac{1}{2}$).

Mr Haldane has kindly calculated for me the theoretical expectations for this progressive alteration of sex-ratio. The details will be found in the appendix to this paper, but the general results may be briefly summarized as follows:

(I) External agency altering the sex-ratio by converting zygotes of male chromosome-constitution into "somatic" females.

(A) Sex-chromosome formula ♀XX, ♂XY.

The decrease in the preponderance of females is slight, and finally comes to a limiting value, which is itself determined by the existence of a limiting value for the proportion of somatic (XY) to genetic (XX) females. During the action of the modifying factor, the sex-ratio will always show a preponderance of females. The preponderance of males after a swing-back is always less than the previous preponderance of females, and always slight; it can never be greater than $\sqrt{2}$. ♂ : 1♀. A definite proportion of non-viable YY zygotes will always be produced, the greatest possible number being slightly over $\frac{1}{7}$ of the total number of zygotes.

(B) Sex-chromosome formula ♀*ZW*, ♂*ZZ*.

In this case, a very different result is produced. There is no limiting value to the proportion of somatic (*ZZ*) females, and the process continues until *all* the females are somatic, the *W*-chromosome being entirely eliminated from the stock. The limiting sex-ratio is therefore the same as the number of *ZZ* organisms transformed into females in any generation. I.e. if this proportion is less than 50 %, the preponderance of females will become converted into a preponderance of males by the action of the modifying factor itself. If the modifying factor does not cease to operate until after all the females have become *ZZ*, the swung-back generation will be all males, and therefore incapable of further existence without the importation of individuals from other stocks. Even after a very few (two or three) generations the swung-back preponderance of males may be considerable, and greater than the previous preponderance of females. No non-viable zygotes will be produced.

(II) If the external factor is of such a kind that it transforms zygotes of female constitution into somatic males, the results given above must simply be transposed, those for the *XX*, *XY* formula now applying to organisms of the *ZW*, *ZZ* type, and *vice versâ*.

The figures recollected by Mr Boulenger make it clear that, if a modifying factor of the kind here postulated was at work in Girardinus, then the sex-chromosome formula for this species must be of the ♀*XX*, ♂*XY* type.

It may be noted that there exists another possible explanation for a swing-back of sex-ratio; viz. the acclimatization or adaptation of the organism to the modifying factor, which would then cease to exert its effect.

It is proposed to carry out some experiments with a view to testing these hypotheses. Meanwhile this note is published with the purpose of drawing the attention of those engaged in genetic research to certain theoretical expectations with regard to sex-ratios.

I must thank Mr E. G. Boulenger for supplying me with unpublished data with regard to Girardinus, and Mr J. B. S. Haldane for his help with the mathematical calculations involved.

To sum up in general terms: If, in any given species, (1) there exists a chromosome-difference between the two sexes which plays a part in the determination of sex; and (2) this determination by means of chromosomes can be modified by the action of other factors; then (3) we shall find organisms with the chromosome-constitution of one

sex, but the actual organization of the other. Such individuals when mated to normal individuals in normal conditions will always give a preponderance of the sex other than that to which they themselves belong. Further, if such an external factor modifying sex-ratio becomes operative for a period of several generations, but is subsequently eliminated, we shall get a decreasing preponderance of the one sex during this period, then a preponderance of the opposite sex in the first generation after the close of the period, and finally the normal sex-ratio in succeeding generations. Abnormal sex-ratios may thus be produced in a great variety of ways:

(1) by differential fertilization;

(2) by differential mortality of gametes;

(3) by differential mortality of zygotes;

(4) by the conversion of an *X* into a *Y* chromosome or *vice versâ* (extremely improbable);

(5) by the overriding of the chromosome-constitution by means of external influences, so that zygotes result with the facies of one sex but the chromosome-constitution of the other.

It should be noticed that wherever there is a swing-back of sex-ratio, we must assume the last of these alternatives. Morgan ('19, pp. 197–198) has already envisaged this. His comment is as follows: "It is perhaps needless to point out that if, in a species in which sex is determined by a chromosome-mechanism, it were possible to change the sex by other agencies in spite of the chromosome arrangement, the latter relation would be entirely thrown out of gear and males would transmit sex-linked characters and sex itself like females, and females like males. As no such cases have been found, it is futile to discuss such a possibility." The case of Girardinus is, however, one where this is the most probable explanation; and it therefore becomes important to test other cases of abnormal sex-ratio for this possibility. In addition, the phenomena in Lymantria also demand this explanation.

APPENDIX.

(I) Calculation of the change in constitution and sex-ratio resulting from the continuance through a number of generations of a modifying factor transforming a proportion of zygotes of male chromosome-constitution into somatic females.

(A) *When ♂ is XY, ♀ is XX.*

Suppose that $\frac{p}{1}$ of the XY zygotes in each generation are transformed into females.

If the constitution of the nth abnormal generation be $u_n\,XY : 1XX$, then $pu_n\,XY$ will be transformed into females, and the sex-ratio will be

$$(1-p)\,u_n\,(XY)\,♂ : 1(XX) + pu_n\,(XY)\,♀ = (1-p)\,u_n\,♂ : 1 + pu_n\,♀.$$

Of the gametes, the spermatozoa will be $1X : 1Y$.

The ova will be $(2 + pu_n)\,X : pu_n\,Y$.

The constitution of the next generation will therefore be

$$pu_n YY \text{ (non-viable)} : (2 + 2pu_n)\,XY : (2 + pu_n)\,XX.$$

Thus $$u_{n+1} = \frac{2 + 2pu_n}{2 + pu_n}, \text{ or, if } q = \frac{1}{p}, = \frac{2q + 2u_n}{2q + u_n} = 1 + \frac{u_n}{u_n + 2q}.$$

Now $$u_1 = 1, \quad \therefore u_2 = 1 + \frac{1}{1 + 2q}, \quad \therefore u_2 > u_1.$$

Similarly $u_3 > u_2$. However, u tends to a limit given by

$$u_\infty = 1 + \frac{u_\infty}{u_\infty + 2q},$$

$$\therefore u_\infty{}^2 + 2\,(q-1)\,u_\infty = 2q,$$

$$\therefore u_\infty = 1 + \sqrt{q^2+1} - q.$$

And the final sex-ratio is

$$\frac{(1-p)\,u_\infty \,.\, ♂}{1 + pu_\infty \,.\, ♀} = \frac{(q-1)\,u_\infty}{q + u_\infty} = \frac{(q-1)\,(1 + \sqrt{q^2+1} - q)}{1 + \sqrt{q^2+1}}$$

$$= \frac{q-1}{q}\,(q + 1 - \sqrt{q^2+1}).$$

When the disturbing factor ceases to operate, the swung-back sex-ratio will be $1\,♀ : u_n\,♂$. The preponderance of males after a swing-back

cannot exceed $\sqrt{2}$ (since q cannot be less than 1), and is always less than the previous preponderance of females. In each generation there will always be a proportion of non-viable YY zygotes (the highest possible figure being a little over $\frac{1}{7}$ of the total zygotes).

A few numerical results may be of interest.

p	Sex-ratio of first abnormal generation ♀ : ♂	Swung-back sex-ratio after first generation ♀ : ♂	Final (limiting) sex-ratio ♀ : ♂	Swung-back sex-ratio after limiting generation ♀ : ♂
$\frac{9}{10}$	1 : 0·05	1 : 1·31	1 : 0·06	1 : 1·39
$\frac{3}{4}$	1 : 0·14	1 : 1·27	1 : 0·16	1 : 1·33
$\frac{1}{2}$	1 : 0·33	1 : 1·20	1 : 0·38	1 : 1·24
$\frac{1}{4}$	1 : 0·60	1 : 1·11	1 : 0·66	1 : 1·12
$\frac{1}{10}$	1 : 0·82	1 : 1·05 –	1 : 0·85	1 : 1·05

Thus the final as well as the initial sex-ratio will always show a preponderance of females.

The proportion of YY zygotes in the second generation will be $\frac{1}{12}$ of the total when $p = \frac{1}{2}$, between $\frac{1}{8}$ and $\frac{1}{9}$ when $p = \frac{9}{10}$. In the generation with the limiting sex-ratio it will be over $\frac{1}{10}$ when $p = \frac{1}{2}$, almost $\frac{1}{7}$ when $p = \frac{9}{10}$.

(B) *When ♂ is ZZ, ♀ is ZW.*

Suppose that $\frac{p}{1}$ of the ZZ zygotes in each generation are transformed into females. If the constitution of the nth abnormal generation be $u_n ZZ : 1ZW$, then $pu_n ZZ$ will be transformed into females, and the sex-ratio will be

$$(1-p)\,u_n\,(ZZ)\,.\,♂ : 1\,(ZW) + pu_n\,(ZZ)\,.\,♀ = (1-p)\,u_n\,♂ : 1 + pu_n\,♀.$$

Of the gametes, the spermatozoa will be all Z.

The ova will be $(1 + 2pu_n)\,Z : 1\,W$.

The constitution of the next generation will therefore be

$$1 + 2pu_n ZZ : 1ZW.$$

Thus $$u_{n+1} = 1 + 2pu_n.$$

Hence u_n increases *without limit*, since $u_2 > u_1$, and the increase is larger in each generation. Hence eventually *all* the zygotes will be of constitution ZZ, and the sex-ratio will therefore become $1-p$ ♂ : p ♀.

Once the final sex-ratio is attained (which will take longer the smaller p is), a subsequent swing-back will give nothing but males. When p is large ($> \frac{1}{2}$), a swing-back after very few generations will give

a large preponderance of males, often larger than the previous preponderance of females. No non-viable zygotes are produced.

p	Sex-ratio of first abnormal generation	Swung-back sex-ratio after first generation	Sex-ratio of third abnormal generation	Swung-back sex-ratio after third generation	Final sex-ratio (all ZZ in constitution)
	♀ : ♂	♀ : ♂	♀ : ♂	♀ : ♂	♀ : ♂
$\frac{9}{10}$	1 : 0·05	1 : 2·8	1 : 0·09	1 : 8·94	1 : 0·11
$\frac{3}{4}$	1 : 0·14	1 : 2·5	1 : 0·24	1 : 6·43	1 : 0·33
$\frac{1}{2}$	1 : 0·33	1 : 2·0	1 : 0·55	1 : 3·50	1 : 1·00
$\frac{1}{4}$	1 : 0·60	1 : 1·5	1 : 0·91	1 : 1·81	1 : 3·00
$\frac{1}{10}$	1 : 0·82	1 : 1·2	1 : 0·98	1 : 1·24	1 : 9·00

Thus, while the initial sex-ratio of course shows a preponderance of females, the final sex-ratio shows a preponderance of males when $p < \frac{1}{2}$.

(II) If the disturbing factor is such as to convert zygotes of female constitution into somatic males (as in Hertwig's frogs), the above calculations must be reversed, those for XX, XY organisms now applying to those of formula ZW, ZZ, and *vice versâ*. The limiting ratio will in this case occur in organisms of the ZZ, ZW type, whereas in those of XX, XY type, the process will continue until only individuals of XX constitution are found.

LITERATURE.

Boulenger, E. G. ('12.) *Proc. Zool. Soc.* 1912 (2), p. 906.

Doncaster, L. ('12.) *Science* (N.S.), Vol. xxxvi. 1912, p. 282.

Gerschler, M. W. ('14.) *Zeitschr. f. Indukt. Abst. u. Vererbungslehre*, Bd. xii. 1914.

Goldschmidt, R. ('16.) *Amer. Nat.* Vol. l. 1916.

—— ('19.) *Biol. Zentralbl.* Bd. xxxix. 1919, p. 498.

Harper, E. H. ('04.) *Amer. Journ. Anat.* Vol. iii. 1904.

Harrison. ('19.) *Journ. Genetics*, Vol. ix. 1919.

Hertwig, R. ('07.) *Verh. d. Deutsch. Zool. Ges.* Bd. xvii. 1907.

Keilin, D. and Nuttall, G. H. F. ('19.) *Parasitology*, Vol. xi. 1919.

King, H. D. ('12.) *Anat. Record*, Vol. vi. 1912.

Kuschakewitsch, S. ('10.) *Festschrift für R. Hertwig*, 1910, Bd. ii.

Lillie, F. R. ('17.) *Journ. Exp. Zool.* Vol. xxiii. 1917.

Morgan, T. H. ('19.) *The Physical Basis of Heredity*, Lippincott, 1919.

Pearl, R. and Parshley, H. M. ('13.) *Biol. Bull.* Vol. xxiv. 1913.

Riddle, O. ('16). *Amer. Nat.* Vol. l. 1916.

Shull, G. H. ('14.) *Zeitschr. f. Indukt. Abst. u. Vererbungslehre*, Bd. xii. 1914.

Smith, G. W. ('06.) *Rhizocephala*, Berlin, 1906. (Fauna u. Flora des Golfes v. Neapel, xxix.)

Steinach, E. ('12.) *Pflüger's Archiv f. d. Ges. Physiol.* Bd. cxliv. 1912.

—— ('13.) *Zentralblatt f. Physiol.* Bd. xxvii. 1913, p. 717.

Strasburger, E. ('00.) *Biol. Centralbl.* Bd. xx. 1900.

—— ('10.) *Jahrb. f. Wiss. Bot.* Bd. xlviii. 1910.

Strong, R. M. ('12.) *Biol. Bull.* Vol. xxiii. 1912.

GENETIC STUDIES IN POULTRY.

II. INHERITANCE OF EGG-COLOUR AND BROODINESS.

By R. C. PUNNETT and the late Major P. G. BAILEY, R.F.A.

(With 13 Text-figures and Plate IX.)

Introductory.

In 1910 we started some experiments with the intention of attempting to work out the genetics of egg-colour and of broodiness in poultry. At the time at which they were begun there was a fair prospect of means being found to carry them to a definite conclusion. This prospect was shattered by the advent of war, and the work was gradually curtailed until eventually it had to be abandoned. Nevertheless, the experiments, fragmentary as they are, present some points of interest which seem worthy of being placed on record.

Few observations concerning the genetics of either egg-colour or broodiness have hitherto been published. Hurst (1905) found on crossing the two non-sitting breeds, White Leghorn and Houdan, that none of the F_1 hens went broody; but that from the cross Black Hamburgh × Buff Cochin the F_1 pullets all went broody like the Cochin. With regard to egg-colour he states that from the former cross all the pullets laid only white eggs, while from the Hamburgh-Cochin cross the F_1 ♀♀ all laid "eggs of varied hue, none being so brown or so white as the parent breeds." Neither character appears to have been the subject of investigation beyond the F_1 generation.

A remarkable account of the mode of transmission of egg-colour was published by Holdefleiss in 1911. He contended, among other things, that the colour of the egg laid by the hen is affected by the cock that fertilises it: that the hens resulting from a cross between birds belonging to brown- and white-egglaying strains lay both brown and white eggs: and that such F_1 hens mated to a brother produce both brown and white eggs in the ratio 3 : 1. Though we regard Holdefleiss' paper as open to destructive criticism we do not consider it necessary here to do

more than state that we have encountered no phenomena of the kind in the course of our work.

Inheritance of Egg-Colour.

The experiments, of which an account is now given, were designed for the investigation of egg-colour and of broodiness on the same material. For convenience the crosses made will be considered first in connection with egg-colour. In 1910 and 1911 F_1 birds were produced from the mating Brown Leghorn ♀ × Black Langshan ♂, and in 1912 from the reciprocal. The Brown Leghorn hens belonged to a strain which had been under observation for some years previously. The eggs were white, and broodiness was never observed. The Langshans were purchased in 1910, and during this season and the following one they were used for breeding pure Langshan as well as for crossing. Those bred all laid brown eggs of a depth similar to that of the egg figured on Pl. IX, fig. 5, though perhaps not quite so pink in tone. The variation in the depth of the colour in the various birds was slight. Subsequently a further set of experiments was made in which the non-broody white egg breed used was the Gold Pencilled Hamburgh, the brown egg broody race being, as before, the Langshan.

At this point a brief digression is necessary in order to explain the procedure adopted in recording the colour of the egg. After consideration of several methods we decided to make use of the standard colour charts published by the Société française des Chrysanthémistes. It was not always possible to match the tint of a given egg exactly, for some had slightly more pink, and others again slightly more yellow than the nearest colour on the chart. Nevertheless we felt that the record was sufficiently accurate for our purpose, since it was the depth of colour rather than the exact tint that we wished to register. Six sheets of the chart, each with four different shades, were made use of, viz.:

Jaune maïs, 36[1].
Saumon jaunâtre, 65.
Chair tendre, 68.
Saumon rougeâtre, 73.
Chair rosé, 136.
Rose de Nymphe, 137.

The sheets were carefully collated by eye and the following 12 grades were established for use in the experiments:

Grade 1. White.
„ 2. Very slightly tinted.
„ 3. R. de N. 1.
„ 4. R. de N. 2.

[1] The number after each colour denotes the number of the sheet in the publication referred to.

Grade 5. R. de N. 2, Ch. t. 1.
„ 6. R. de N. 4, Ch. t. 2, Ch. r. 1, J. m. 1.
„ 7. Ch. t. 3, Ch. r. 2.
„ 8. Ch. t. 4, Ch. r. 3.
„ 9. Ch. r. 4, J. m. 2.
„ 10. J. m. 2, S. roug. 1, S. jaun. 1.
„ 11. J. m. 4, S. roug. 2, S. jaun. 2.
„ 12. S. roug. 3, S. jaun. 3.

These grades are of course arbitrary, and in passing from the lowest to the highest there is no regular quantitative accession of colour. The difference between grades 2 and 3 for example is certainly much less than between grades 9 and 10. Where the amount of colour is slight a difference is more easily apprehended than where the shade is deeper.

In practice a difficulty arises from the fact that the tint of a given hen's eggs often varies to some extent during her laying period. Our experience has been that the eggs laid earlier in the series are generally rather darker than those laid later on in the season, and in the case of the more deeply tinted eggs there may even be a difference of several grades between those laid in March and those laid in July. Sometimes also the first few eggs laid by a pullet are variable in tint. Our practice therefore has been to neglect the first few eggs and to base our record on several eggs laid in the earlier part of the season. Our experience has been that, in the great majority of cases, such eggs show practically no variation in tint.

In the case of a few birds a further difficulty has arisen in attempting to grade the egg. In these cases a tinted egg is laid which has the appearance of being covered with a delicate chalky film. That the texture of the shell plays a part in the tint of the egg is obvious, but the extent to which the two are interdependent would necessitate research directed upon this special point. In the case of these "chalky" eggs we decided, after some hesitation, to record their grade as they appeared when moistened and rubbed. After such treatment the egg appears several grades darker than when fresh from the oviduct.

We may now turn to the experimental results, taking first the Leghorn-Langshan cross. Originally this was made between a Leghorn ♀ and a Langshan ♂. The Leghorn belonged to the strain procured by Mr Bateson from Mr Boys-Smith in 1900–01. Even at that time the birds were closely inbred[1]. The strain was kept pure from admixture with any other strain until it was used in 1910–11 for crossing with the Langshan. By that time hens alone remained. Though large numbers of chicks were hatched it was found impossible to raise a cockerel. The healthiest survived until about one-third grown and then succumbed to sudden and mysterious fits. The majority died soon after hatching. Pullets were more easy to rear, though even here the mortality was considerable. Those reared were slow growers, on the small side, and

[1] Cf. Bateson and Saunders, 1902, p. 92, where a pedigree is given.

with that delicacy of appearance so often characteristic of the highly inbred thing. On being crossed with the Langshan ♂ the chicks left nothing to be desired from the point of view of health and vigour. The black down of the Langshan proved to be dominant over that of the brown stripe of the Leghorn, and a simple 3:1 ratio was realised in F_2. In adult plumage the F_1 hens were full black with dark eyes and shanks; the F_1 cocks were, on the whole, black with a varying amount of orange in the neck, shoulder and saddle hackles: the eye was orange and the shanks were horn on light. The sexual differences in the last two characters are doubtless due to a sex-linked inhibitor introduced by the Brown Leghorn hen. The eggs laid by the F_1 birds were tinted, though rather lightly, approximating to grades 4 and 5[1]. In 1911–12 a number of F_2 ♀ ♀ [2] were reared, and 65 were subsequently tested in respect of egg-colour. The results are shown schematically in Fig. 1. The range is from white up to grade 10, i.e. almost as deep as the Langshan's eggs. The great majority however laid white or light-tinted eggs.

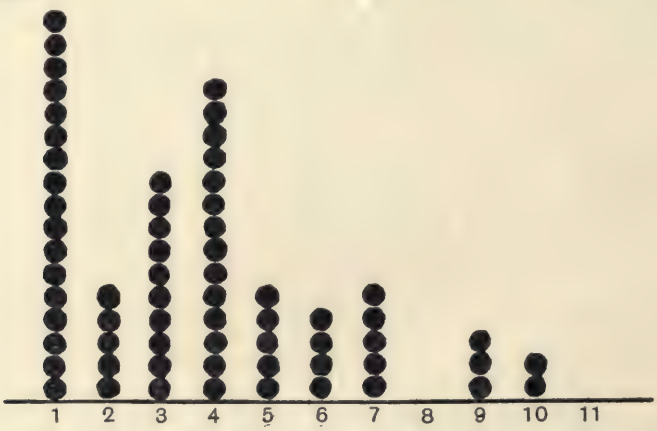


Fig. 1. Distribution of grades of egg-colour in F_2 generation ex Brown Leghorn ♀ × Langshan ♂.

Before passing on to the Hamburgh-Langshan cross, a further experiment with the Brown Leghorn and Langshan calls for attention. In 1912 we made the cross reciprocal to that described above, mating a Brown Leghorn ♂ with a Langshan ♀. The Leghorn ♂ was purchased,

[1] This statement is from recollection. The original records of the F_1 birds were in the keeping of my colleague. In the turmoil of war they appear to have gone astray, and I was unable to trace them among his papers after he had been killed. [R. C. P.]

[2] 3 F_1 ♂ ♂ and 12 F_1 ♀ ♀ in all were used for producing the F_2 generation.

and there is no reason for supposing that it was in any way related to our original stock. Of the F_1 birds the cockerels were very similar to those bred from Brown Leghorn ♀. The pullets however had orange eyes, though often with a few small patches of pigment, and their shanks were horn on light instead of horn on slaty. This is doubtless due to the Leghorn ♂ having been homozygous for the inhibitor for which we found the Leghorn ♀ heterozygous. Of interest here is the fact that the distribution of egg colours in the F_2 pullets[1] raised from this cross was quite different from that found in the reciprocal. As Fig. 2 shows

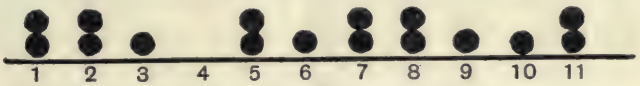


Fig. 2. Distribution of grades of egg-colour in F_2 generation ex Langshan ♀ × Brown Leghorn ♂.

the range is complete, from white to grade 11; but there is no excess of white and light tinted eggs as in the previous case. The mean grade is higher here than in Fig. 1, viz. 6·0 as compared with 3·7. It should be stated that the F_1 ♀♀ ex Langshan ♀ laid a more deeply tinted egg than the F_1 ♀♀ ex Brown Leghorn ♀.

The F_2 generation ex Langshan ♀ × Hamburgh ♂ forms an interesting commentary on the Leghorn-Langshan results. Eggs laid by F_1 pullets were tinted, belonging to about grades 6—7 (cf. Pl. IX. fig. 6). The distribution of egg-colour among the 51 F_2 pullets[2] is shown in Fig. 3. There is a complete range from the white of the

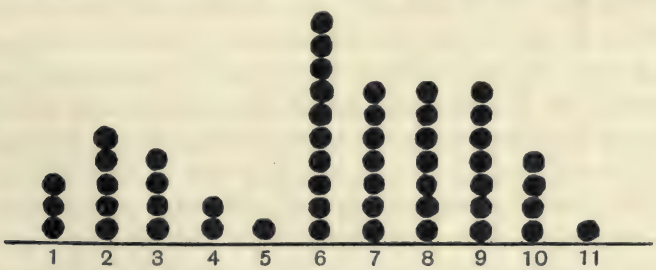


Fig. 3. Distribution of grades of egg-colour in F_2 generation ex Langshan ♀ × Hamburgh ♂.

Hamburgh up to the deep colour of the Langshan. The curve is evidently bimodal, and can readily be separated into two portions—a smaller one in which the colours range from 1—5, and a larger in which the range

[1] Raised from 2 F_1 ♀ ♀ and 1 F_1 ♂.

[2] Raised from 2 F_1 ♂ ♂ and 3 F_1 ♀ ♀.

is from 6—11. Those belonging to the more deeply tinted group are about thrice as numerous as the white and faintly tinted ones.

These are the chief facts with regard to egg-colour that have come from our researches, and before passing on to some special points we may consider their possible orientation. The data are clearly insufficient to justify any attempt at precise factorial analysis. Such analysis as we can pretend to must obviously be rough, and our only excuse for making it is that it may perhaps offer hints for future work to others.

We suggest then that the cases of the Langshan ♀ × Brown Leghorn ♂ (Fig. 2) and of the Langshan ♀ × Hamburgh ♂ (Fig. 3) are on the same plane, and may be taken together (Fig. 4)—that we are

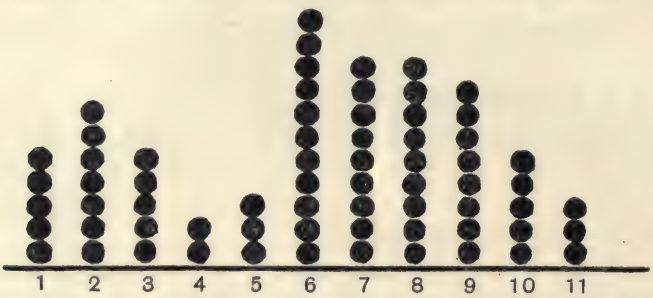


Fig. 4. In this figure the data given in Figs. 2 and 3 are combined.

concerned here with a principal factor leading to increased tint of egg—that this factor is present in a homozygous state in the Langshan, but absent from the Hamburgh and the Brown Leghorn—that in the heterozygous state the tint is intermediate. Further we suggest that the egg tint is also dependent upon one or more minor factors, which are present in the Langshan but not in the Hamburgh or the Leghorn. The effect of their presence is to lead to tinted shell, but only to a minor degree. The really dark egg must owe its colour to the principal factor, but the colour will be deeper if the minor factors are also present.

Our suggestion may perhaps be made clearer if we attempt to illustrate it by a concrete example. Let us suppose the dark egg breed to be homozygous for a major, and for one or more minor factors, while the white egg breed with which it was crossed contains none of these factors. If the various factors are inherited alike by either sex the distribution of egg-colour in the F_2 generation will be not unlike the scheme shown in Fig. 5. One-quarter of the F_2 birds will lack the major factor, and their eggs may be supposed to range between grades 1—4 according to their constitution with regard to the minor factors. The

other three-quarters will contain the major factor and will vary between grades 5—11 according as this major factor is associated with one or more of the minor factors. Moreover the depth of tint may further depend upon whether the bird is homozygous for the major or for the minor factors. In this way we can form a rough interpretation of the nature of the F_2 generation such as that shown in Fig. 4.

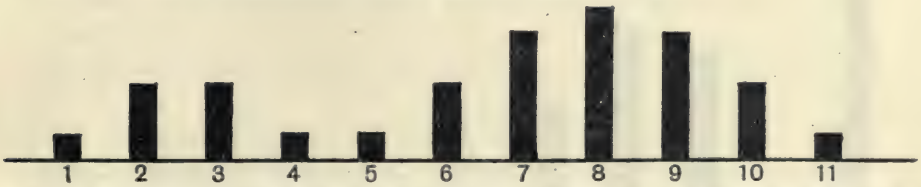


Fig. 5. Ideal scheme of distribution of grades of egg-colour in F_2. For explanation see text.

We have already seen however that the F_2 curve of egg-colour for the birds ex Brown Leghorn ♀ × Langshan ♂ is of quite a different type (Fig. 1), and is characterised by the enormous preponderance of white and of lightly tinted eggs. Now a curve of such a nature can be readily formed from our ideal curve (Fig. 5) if we suppose that the F_1 birds are of similar constitution with regard to the factors leading to pigment production, but at the same time are heterozygous for a factor leading to the inhibition of pigmentation in the eggs. Suppose that this inhibitor turns grades 1—6 into grade 1, grade 7 into grade 2, 8 into 3, 9 into 4, 10 into 5, and 11 into 6: and suppose further, for the sake of simplicity, that its action is similar whether the bird be homozygous or heterozygous: then our ideal curve shown in Fig. 5 is transformed into the curve shown in Fig. 6. Without harrying the point too closely it is clear that such a curve bears a fair resemblance to the actual curve met with in Fig. 1. If we accept this interpretation we must suppose that the Boys-Smith strain of Brown Leghorn was homozygous for this postulated inhibitor, which was lacking in the purchased Brown Leghorn ♂ and in the Hamburgh. There is the point of course that where the white egg breed was the mother one type of F_2 family appeared, and that where it was the father another type appeared: but in the absence of further data it seems hazardous to regard this as anything but a coincidence.

Though the interpretation which we have suggested is in fair accordance with the experimental data so far given, there is one further set of experiments which offer a difficulty to that interpretation. The two F_1 ♂♂ ex Langshan × Hamburgh were crossed with several Hamburgh ♀♀. Since such F_1 ♂♂ mated with similarly bred F_1 ♀♀

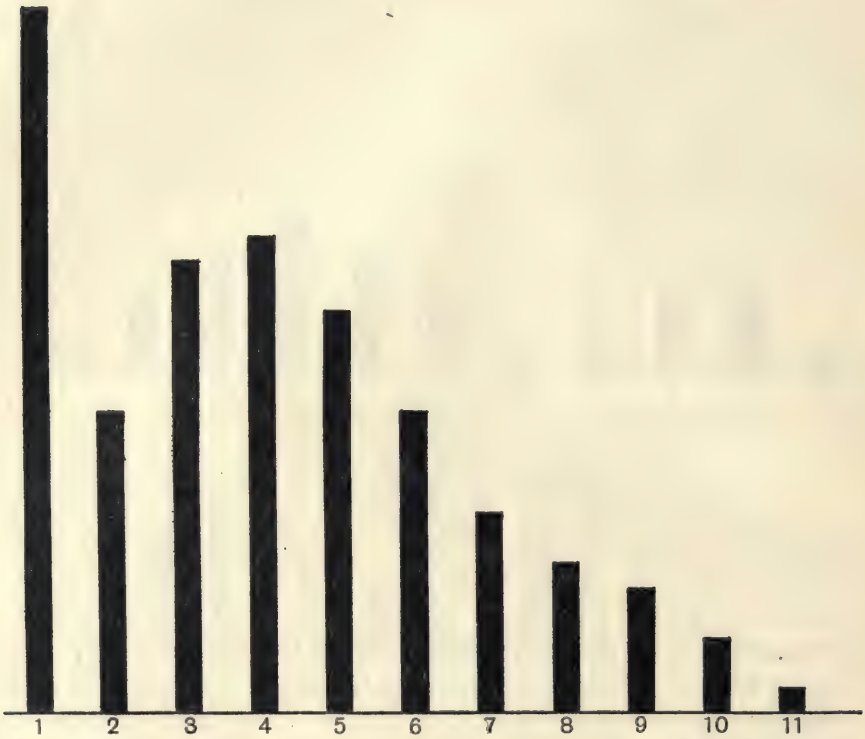


Fig. 6. Illustrating the alteration in the nature of the curve presented in Fig. 5 when the F_1 birds are supposed to be also heterozygous for a factor inhibiting the production of pigment in the egg-shell.

gave a bimodal curve (Fig. 3) we should look for a bimodal curve also when such males are mated with Hamburgh ♀♀; though we shall of course expect both modes to be shifted in the direction of lower grade. The curve actually obtained is shown in Fig. 7, and is certainly not

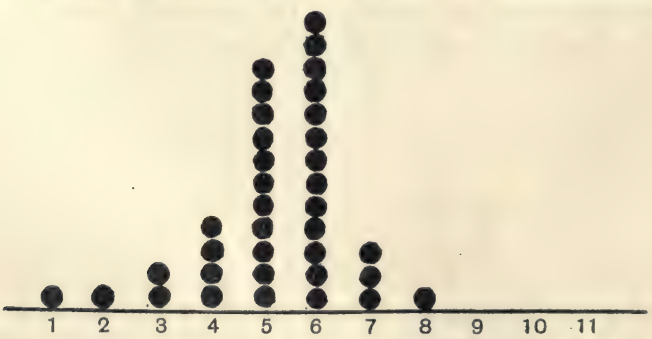


Fig. 7. Distribution of grades of egg-colour from F_1 Hamburgh-Langshan ♂♂ × Hamburgh ♀♀.

bimodal. A higher mode at about grade 6 was to be expected, but so also was another one at about grades 2—3. It is clearly not there and we cannot account for its absence.

Egg-colour and down-colour.

A. In the Brown Leghorn-Langshan cross.

We have already mentioned that in the Langshan and Leghorn cross the brown-striped down behaves as a simple recessive to the black. F_2 pullets black in the down develop for the most part into full blacks, though some may show a little gold in the hackle, and a little brown mossing in the wings. The brown-striped F_2 chicks feather up into various types of brown, a bird not far removed from the Dark Dorking type being perhaps the most common. The typical Brown Leghorn reappears but rarely. Of the 65 F_2 ♀♀ tested for egg colour ex Brown Leghorn ♀ × Langshan ♂ (Fig. 1) 19 were brown-striped in the down, and 46 were black. If the two down classes are considered apart a remarkable difference is noticeable in the distribution of the egg tints. For nearly all of the birds laying a white or almost white egg belong to the blacks (Fig. 8), eggs of such tint being but rarely found among the browns (Fig. 9). At first sight it seems tempting to

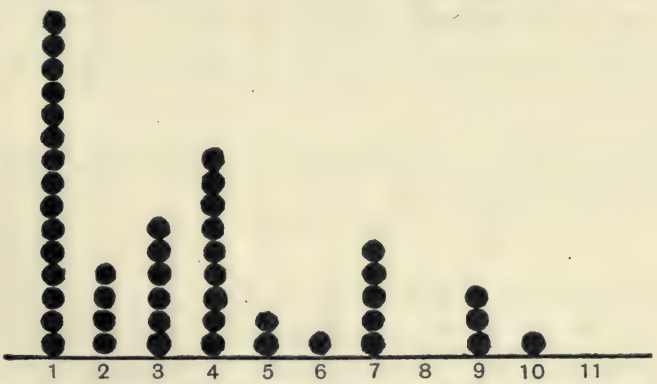


Fig. 8. Distribution of grades of egg-colour in the black F_2 birds ex Brown Leghorn ♀ × Langshan ♂.

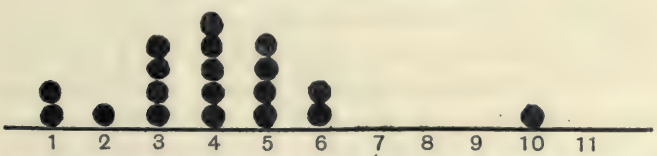


Fig. 9. Distribution of grades of egg-colour in the brown F_2 birds ex Brown Leghorn ♀ × Langshan ♂.

suggest that there is some form of linkage between the factor for black down and that for our postulated inhibitor of egg-colour. But when it is recollected that the inhibitor originally entered the cross from the side opposite to that from which the black entered, it is clear that we cannot be dealing with an ordinary case of linkage.

B. In the Hamburgh-Langshan cross.

The down-colour of the Gold Pencilled Hamburgh is pale golden with dark brown spotty markings on the back of the head, and generally also on the body. The down-colour of F_1 birds is that of blacks. In the F_2 generation four main types of down could be distinguished, viz. black, brown stripe, pale gold (similar to that of the Hamburgh), and chocolate. The last mentioned exhibited considerable variation. The predominant colour was always rich chocolate, but in some cases the chick could be described as a chocolate stripe, while in others the colour was uniformly distributed, and in others again there might be a slight admixture of black. Chick black in down developed into blacks: the pale golds developed into gold barred birds of various sorts: the brown stripes into a miscellaneous lot of browns and gold browns. The chocolates however gave two distinct classes of plumage, viz. gold barred of various kinds, and blacks, these last always with some brown mossing in the wings or else with some gold in the hackle[1]. No full black was actually reared from a chocolate chick though this may have been due to paucity of numbers. Of 31 chicks with chocolate downs reared 11 developed into blacks.

Out of the four kinds of down the actual numbers recorded for the several F_2 families were

Blk.	Choc.	Br. Str.	Gold
96	50	31	16

But if we reckon one-third of the chocolates as potential black-plumaged birds our ratio for the plumage characters, black as opposed to non-black, becomes

Black	Non-Black
96 + 17 = **113**	33 + 31 + 16 = **80**

This is very close to a 9 : 7 ratio where expectation would be 109 blacks: 84 non-blacks. The conclusion that the Hamburgh lacks two factors necessary for black is supported by the results of crossing F_1 birds with Hamburghs.

[1] These descriptions refer throughout to the pullets. For reasons of economy the cockerels were killed off at an early stage.

The four types of down appeared as follows:

Black	Choc.	Br. Str.	Gold
17	36	30	38

Reckoning one-third of the chocolates as blacks our ratio of blacks : non-blacks in respect of plumage becomes $17 + 12 = 29 : 92$. The blacks form almost exactly the quarter of the total that theory demands.

Where black plumage depends upon two factors, as in this cross, it is clear that an association between egg tint and plumage colour would be less easy to demonstrate statistically, even should it occur. In Figs. 10 and 11 the F_2 blacks and non-blacks have been set out with reference

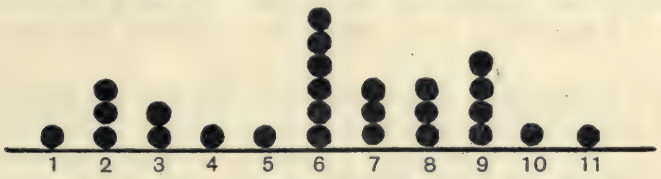


Fig. 10. Distribution of grades of egg-colour in black F_2 birds ex Langshan ♀ × Hamburgh ♂.

Fig. 11. Distribution of grades of egg-colour in brown and gold birds of F_2 generation ex Langshan ♀ × Hamburgh ♂.

to the egg-colour. So far as they go the two curves are obviously of a similar nature, and there is no reason for supposing that any factor influencing egg-colour is here associated with blackness of the plumage.

Broodiness.

Our data regarding broodiness are less satisfactory than those concerned with egg-colour. The character is a difficult one to classify. Between the obviously broody bird that will sit regularly each year and the equally obviously non-broody that never shows the least inclination to sit, there are many grades, as indeed is well known. In the earlier part of our experiments we kept the birds during their pullet year, and classified them as non-broody if they showed no tendency to sit during that period. After their first year we got rid of all the birds tested through lack of space. Only after several years did we realise that a bird might show no inclination to sit during her pullet year, and yet might become a reliable broody the following season. We have also

encountered cases where a bird might go broody in her pullet year, and yet show no sign of broodiness the following season.

The material was the same as that used in the egg-colour work. Both the Brown Leghorn and the Hamburgh strains were under observation for some years and in neither of them did we ever observe any sign of broodiness. The broodiness of the Langshan we took on trust when we purchased our original birds. Such hens as we bred and had under observation went broody, though we never actually made use of them for sitting.

As in the case of egg-colour we found a marked difference in the Hamburgh × the Brown Leghorn crosses. F_1 birds ex Brown Leghorn ♀ × Langshan ♂ went broody, and this was also true of the reciprocal.

In the F_2 birds the distribution of broody and non-broody was as follows:

	Broody	Non-Broody
Ex Brown Leghorn ♀ × Langshan ♂	19	47
Ex Langshan ♀ × Brown Leghorn ♂	8	8

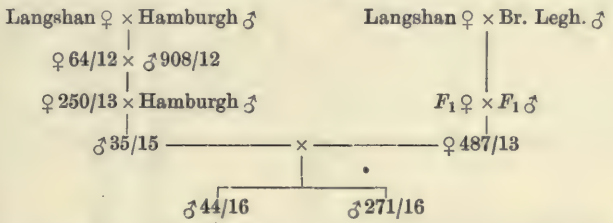
The birds were kept only during the pullet year and had we been able to keep them for another season it is possible that the proportion of broody birds would have been increased.

The data from the Hamburgh cross are of very different type. Three F_1 ♀ ♀ were used, of which one, ♀ 64/12, never went broody over three seasons, while the other two (♀ 453/13 and ♀ 454/13) only showed slight signs of broodiness. Two ♂♂ were used, viz. ♂ 908/12 and ♂ 452/13. Presumably both carried some factor for broodiness since ♂ 908/12 gave broody daughters with the non-broody ♀ 64/12 and ♂ 452/13 gave a broody daughter with pure Hamburgh ♀ ♀. As is shown by the figures set out below the proportion of broody birds arising from the Langshan × Hamburgh cross is very much lower than that from the Leghorn × Langshan cross.

		Broody	Non-Broody
F_2 generation	♀ 64/12 × ♂ 908/12	2	2
	♀ 453/13 × ♂ 908/12	1	19
	♀ 454/13 × ♂ 452/13	1	13
F_1 × Hamburgh	♀ 64/12 × Hamburgh ♂	—	8
	Hamburgh ♀ × ♂ 908/12	—	6
	Hamburgh ♀ × ♂ 452/13	1	21

The small proportion of broody birds produced from the Hamburgh cross is also shown by the following experiment. One of the two non-broody birds of the F_2 generation from ♀ 64/12 × ♂ 908/12 was ♀ 250/13, a hen which was full black in plumage, laid a deeply tinted egg (Pl. IX.

fig. 5), and was never observed to go broody during the four years she was kept. Mated with a Hamburgh ♂ this hen produced among other birds ♂ 35/15. He was mated with ♀ 487/13, a black broody F_2 ex Langshan ♀ × Brown Leghorn ♂, and a layer of deeply tinted eggs (Pl. IX. fig. 2)[1]. Two of the male offspring, as shown in the accompanying pedigree, were mated with hens belonging to a pure non-broody strain[2].



Of the daughters of ♂ 44, 13 were tested for broodiness, and all were found to be non-broody. Of the 16 daughters of ♂ 271 that were tested two became broody. This cock, with a father extracted from the Hamburgh cross and with a broody mother, recalls in its behaviour the F_1 Hamburgh-Langshan. For both of them appear to produce but a small proportion of gametes which give rise to broody daughters. We are inclined to think that ♂ 44 was of the same nature, and that he would have produced an occasional broody daughter had it been possible to rear more birds from him. It is tempting to suggest that the Hamburgh may have been carrying a factor, or factors, tending to inhibit the development of the broody habit; but to decide this point far more ample experiments are required.

Mention may be made here of some experiments undertaken with the Silky fowl in which this breed was crossed with the Brown Leghorn (Bateson and Punnett, 1911). The F_1 birds, as is usual in Silky crosses, were all markedly broody, nor did there appear to be any difference between those that had a Silky mother and those whose father was a Silky. Of about 24 F_2 pullets which were kept for a year or more all except one became broody. This low proportion suggests that the manifestation of broodiness, particularly where the character is well marked, may depend upon more than one factor. Should this turn out

[1] The experiment was part of a series designed to establish a non-broody strain laying a deeply tinted egg. Owing to conditions brought about by the war it had to be abandoned though we have little doubt from our general experience that the object could have been achieved.

[2] Extracted from a Hamburgh-Brown Leghorn cross, and belonging to a strain in which no sign of broodiness ever appeared.

to be the case the evidence from the Silky suggests that either factor, if two are concerned, is capable by itself of giving rise to broodiness.

We do not know of any instance in which broodiness has arisen through the crossing of two strains which experience had shown to be pure for the non-broody character. In the course of our experiments during the past ten years we have tested considerable numbers[1] of hens arising from complex crosses between the three non-broody breeds, Pencilled Hamburghs, Sebrights, and Brown Leghorns. Not a single bird has appeared which showed the slightest sign of broodiness. Such data as we have accumulated afford no grounds for the suggestion that the broody habit may depend upon the meeting of complementary factors[2].

Broodiness and dark eggs.

It is well known that most of the races of poultry that lay dark eggs belong to the broody section, whereas the non-broody strains lay white eggs. These facts suggest that there may be some form of linkage between egg-colour and broodiness, a point upon which we hoped that our experiments would throw some light. Figs. 12 and 13 show the

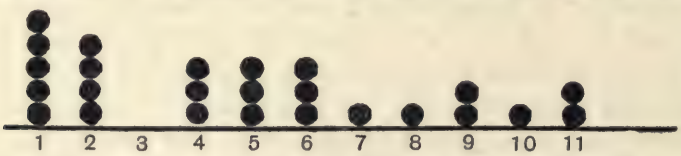


Fig. 12. Distribution of grades of egg-colour in the F_2 broody birds ex Brown Leghorn ♀ × Langshan ♂.

distribution of egg-colour in relation to broodiness in the Langshan-Leghorn cross. For the non-broody birds the average grade of egg-colour is 3·8, while for the broody ones it is 4·9. The numbers are however too few to lay any stress on this small difference. It is conceivable that one of the factors upon which egg-colour depends may be linked with broodiness, and that the slightly higher grade of colour among the broody birds is significant. Nor must it be forgotten that the non-broody birds were, with few exceptions, only tested during their pullet year. Some of them were doubtless potentially broody, and if

[1] Certainly more than 200.

[2] Broody birds, as is well known, may appear in breeds which are nominally non-broody, e.g. White Leghorns. We are inclined to think that in such cases the cock is responsible. After an occasional cross with a broody race it is not enough to rebuild the strain solely by selection of non-broody hens. The cock may also carry broodiness, and should be carefully tested before he is put into the breeding pen.

among these were the few layers of the darker eggs, the difference in grade between the two classes would become much more marked. It is to be regretted that the layers of darker eggs among the birds recorded as non-broody were not more thoroughly tested for broodiness, by

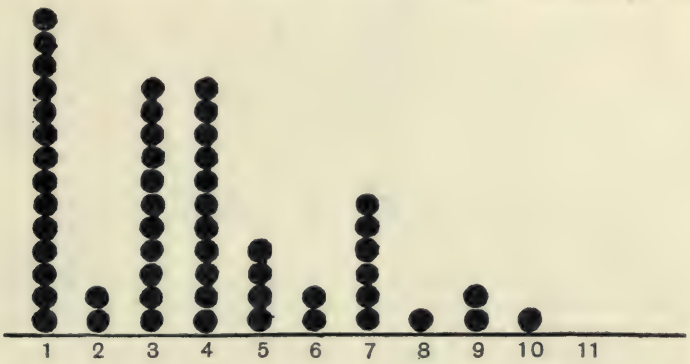


Fig. 13. Distribution of grades of egg-colour in the F_2 non-broody birds ex Brown Leghorn ♀ × Langshan ♂.

keeping them for several years, and we hope that this will be done by anyone who undertakes experiments on similar lines.

That the deeply coloured egg may be laid by a non-broody hen is clear from the case of ♀ 250/13, an F_2 bird ex Langshan × Hamburgh (cf. p. 288). But in this case there are some grounds for supposing that the Hamburgh may carry a factor inhibiting the broody instinct. It is possible that ♀ 250 was potentially broody, and that the manifestation of the instinct was prevented by an inhibitor factor. It is clear however that in practice it would be possible to establish a strain of non-broody birds laying dark coloured eggs.

SUMMARY.

1. F_1 birds from a cross between a white egg laying and a dark egg laying strain lay eggs of an intermediate tint.

2. Segregation occurs in the F_2 generation, the two parental grades, together with all intermediate ones being found.

3. There is evidence for the existence of an inhibitor of pigmentation in one of the white egg strains used, and there is some evidence for regarding this inhibitor as being associated with the factor for black plumage.

4. Broodiness is a highly complex character. F_1 hens are generally broody. The proportion of broody to non-broody birds in F_2 varies greatly in different crosses.

5. The factors for broodiness and for egg-colour are transmitted by the cock as well as by the hen.

6. Though there is slight evidence of some association between the brown egg and the broody habit, the two characters may be dissociated. It is clearly possible to establish a non-broody strain laying brown eggs.

EXPLANATION OF PLATE IX.

Fig. 1. Egg of Brown Leghorn.
Fig. 2. Egg of F_2 ♀ (487/13) ex Langshan ♀ × Br. Legh. ♂. Classed as grade 11.
Fig. 3. Egg of F_1 ♀ ex Br. Legh. ♀ × Langshan ♂. Classed as grade 5.
Fig. 4. Egg of Hamburgh ♀.
Fig. 5. Egg of F_2 ♀ (250/13) ex Langshan ♀ × Hamburgh ♂. Classed as grade 11.
Fig. 6. Egg of F_1 ♀ ex Langshan ♀ × Hamburgh ♂. Classed as grade 6.

LITERATURE.

BATESON, W. and SAUNDERS, E. R. *Reports to the Evolution Committee of the Royal Society.* Report I. 1902.

BATESON, W. and PUNNETT, R. C. "The Inheritance of the Peculiar Pigmentation of the Silky Fowl." *Jour. Gen.* I. 1911.

DAUTHENAY, H. *Repertoire des Couleurs*, publié par la Société française des Chrysanthémistes et René Oberthür avec la collaboration de Henri Dauthenay. 1905.

HOLDEFLEISS, P. "Versuche über Xenienbildung und Vererbungsgesetze bei der Kreuzung von Hühnern." *Ber. phys. Lab. u. Versuchsanst. landwirt. Inst. Univ. Halle*, 1911.

HURST, C. C. *Reports to the Evolution Committee of the Royal Society.* Report II. 1905

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THE GENETICS OF THE DUTCH RABBIT—A REPLY.

By W. E. CASTLE.

PROFESSOR Punnett has honoured me by reviewing in this Journal[1] my paper on the inheritance of white spotting in Dutch rabbits. His own studies of the same subject have led him to suggest an interpretation of the results, which I have reported, somewhat different from my own. I wish to point out that the two interpretations differ less in substance than the casual reader might suppose, and to indicate what are the essential differences and why preference should be given to one interpretation rather than the other.

Professor Punnett has described accurately the essential facts observed. Three races of Dutch rabbits were found to breed true (within limits), each to a different modal condition of the same general type of white spotting. Punnett himself has observed three similar races, though they were perhaps not identical with mine. Upon crossing the races one with another, or with unspotted rabbits, F_1 individuals were obtained which were either intermediate in amount of white spotting or more nearly resembled the darker parent. In F_2, segregation of the original types occurred, but with evidences of modification, the recovered types been less divergent than those which entered the cross. So much as to the facts agreed upon. Now as to the interpretation, I have assumed that the segregation is of a monohybrid sort involving in each cross one principal pair of allelomorphic factors and that the "mutual modification" of types seen in F_2 is due to "residual heredity." Punnett objects to the assumption of modification and to the term residual heredity, unless it is understood in both cases that factorial recombination is meant rather than factorial modification. I am quite ready to concede the point. I used the expression "residual heredity" to cover the unanalyzed genetic agencies responsible for the observed modification in the extracted types. If Professor Punnett prefers to employ the term "genetic factors" I am quite willing to use that phraseology. Punnett

[1] Vol. IX, p. 303.

and I are agreed in supposing that the "residual heredity," when analyzed, will probably be found to be factorial. He refuses to consider any other possibility; I have preferred to use a non-committal term, until a fuller experimental analysis has been made.

The chief difference between Punnett's views and mine lies in the *number* of factors assumed to be in operation in the various crosses and the relative importance of these factors. I have assumed that *one* Dutch factor was present in each race and that the slight genetic variation seen in each uncrossed race, and also the modifications resulting from crossing, were due to minor or modifying factors (collectively "residual heredity"). Punnett ignores genetic variation in uncrossed types and the possibility of modification through minor genetic factors. He assumes that three independent major factors are concerned in the production of Dutch patterns. I have assumed the existence of three allelomorphic conditions of one and the same factor (one in each race), whereas Punnett assumes the existence of three independent factors which in different combinations produce the three races. It should not be difficult to test the relative merits of these assumptions by experiment, for on my view, when two races of Dutch are crossed, monohybrid inheritance ratios are to be expected (3 : 1 in F_2, 1 : 1 in back-crosses), but on Punnett's view dihybrid or trihybrid ratios are often to be expected. If with this point in mind the reader will examine my Text-figure 1[1], in which are summarized the data on crosses between two of the Dutch races studied (viz. Dark and White), I think he will be convinced that the inheritance is unmistakably monohybrid. Punnett does not attempt to apply in detail to this cross his three-factor hypothesis. If he did, I think that hypothesis would be found to fail completely. This cross is, of all those made, least affected by minor genetic factors ("residual heredity"), since the two races crossed were originally isolated from the same stock and may thus be supposed to have had most minor genetic factors in common.

Punnett offers three specific objections to the interpretation of this case as involving a single pair of (major) allelomorphic factors. Each of these objections rests on a misapprehension of the facts.

1. The first objection is that the "Dark" race is not homozygous (in major factors) because too variable. In reality the variation curve for the uncrossed Dark race is steep, monomodal, and nearly symmetrical. It is based on 172 carefully graded progeny all by the same sire. It is the smoothest variation figure of those obtained for any of the Dutch

[1] Carnegie Inst. Wash., Publ. No. 288.

races studied. If there were two major factors involved in this group and part of the mothers were, as Punnett suggests, heterozygous, the variation curve should show two distinct peaks, which it does not. Punnett admits the genetic purity of the White Dutch race with which Dark was crossed, although the total number of individuals is smaller and the variation figure is one-sided. The evidence for the genetic purity of the Dark race is even stronger.

2. The second objection is that my one-factor hypothesis calls for the recurrence in F_2 of the same types as occurred in P_1 and F_1 but not for darker animals, whereas Punnett's hypothesis calls for the occurrence through factorial recombination of darker animals ("a small proportion of animals tending towards complete pigmentation"). Now the actual result here confirms my view and disproves Punnett's. Not a single animal in F_2 was *darker* than animals of the uncrossed Dark race, and the proportion of low-grade Dark animals was less among the extracted than among the uncrossed Darks. I think Punnett must have had in mind the F_2 of a different cross when he raised this point, which is really decisive in favour of my view.

3. The third objection is that, if Dark and White are allelomorphs, when F_1 is back-crossed with White, "the two groups resulting should be sharply separated and their mean grades should be close to those of the parental stocks." Such is actually the case. Punnett apparently is under the misapprehension that the segregated groups, on my hypothesis, should be pure Dark and pure White respectively, whereas they should be (aside from modifications due to minor factors) similar to F_1 and pure White respectively. For if the heterozygote, DW, is back-crossed with WW, it is obvious that the two classes of zygotes to be expected are DW (not DD) and WW respectively. As regards the size of the two segregated groups, they number 65 and 64 respectively with one individual in doubt and not assigned to either group. Certainly this is a very good 1 : 1 ratio. As regards the point that the "mean grades should be close to those of the parental stocks," the mean grade of one group was 7·04 (as compared with 7·28 for F_1) and that of the other group was 15·56 (as compared with 16·25 for uncrossed White, and 14·40 for White extracted in F_2). These are surely very good agreements. In range the two groups agree exactly with the ranges respectively of F_1 and of White extracted in F_2. The groups moreover are monomodal as well as equal in size. In short there is no single criterion for monohybrid Mendelian segregation which is not perfectly met by this case. On the other hand if we apply a two-factor or three-factor scheme to the case,

as suggested by Punnett, we should expect segregation of more than two groups, or the formation of multimodal groups, with extended ranges resulting from recombination of factors, none of which are in evidence.

Punnett attempts a detailed application of his three-factor hypothesis to only one cross, that in which the parent races differ most widely, one race being non-Dutch and entirely devoid of white (the Self race), the other being the whitest of all the Dutch races, the so-called White race. F_1 from this cross consisted of animals bearing a very small amount of white. In F_2 the Self type reappeared in 21·4 per cent. of the young (expected on a monohybrid basis 25 per cent.). The F_1 type (of grades 1—3) was found in 46·6 per cent. of the F_2 young (expectation on a monohybrid basis 50 per cent.). The remaining 32 per cent. of the individuals are found in the range of the White race (where, on a monohybrid basis, we should expect 25 per cent. to occur, if segregation is perfect) or in the region between the ranges of F_1 and uncrossed White. The excess of 7 per cent. over expectation in this group is readily accounted for, if we explain the individuals in the intermediate region between the range of uncrossed White and of F_1, as an assemblage of modified Whites and of modified F_1's respectively. If this is so, a 1 : 2 : 1 ratio is approximated. The data present no serious difficulty to a simple monohybrid explanation, but to make sure of the soundness of such an interpretation a back-cross was made with the White race. This shows a beautiful segregation into two sharply separated groups, each of 58 individuals distributed in a monomodal and substantially symmetrical figure. The range of the lower group is exactly that of the original F_1 group plus the intermediate region in which the doubtful individuals of the F_2 distribution occurred. The range of the upper group is exactly that of uncrossed White plus two additional lower grades (13 and 14) of the intermediate region. Three unoccupied grades (10—12) separate the two groups. It would be difficult to imagine a clearer demonstration of simple monohybrid segregation, if it be granted either that the segregating factors are mutually modified (an hypothesis which I no longer entertain for reasons explained in my former paper) or if it be granted that slight modification of the extracted types has resulted from recombination of minor (modifying) genetic factors in association with the single pair of major factors.

As an alternative to this explanation Punnett has worked out in detail a three-factor hypothesis, which regards the Self race as homozygous for three dominant factors, *P*, *T*, and *S*, the White race being

homozygous for the recessive allelomorphs of these factors. If the three factors were supposed to be equivalent one with another in their whitening influence, the hypothesis would straightway break down in the face of the experimental results. But Punnett adds to the hypothesis as follows. The potency of each of the three assumed factors is supposed to be different. The recessive factor, *p*, is supposed to produce white spotting of the Dutch type either in a heterozygous or in a homozygous state. The recessive factor, *t*, by itself produces a white pattern only in a homozygous state, but in association with *p* or with *s* adds to the whitening effect, raising the grade of the pattern produced.

The third assumed recessive factor, *s*, can by itself produce no white, either as a heterozygote or as a homozygote, but when associated with *p* or with *t* it adds to the whitening effect of those factors in three out of six combinations shown in Punnett's Fig. 2.

This is a rather complicated set of hypotheses to make "out of whole cloth," but no objection need be raised on that score. Any hypothesis is justifiable which will work. But the more secondary hypotheses are required to make it work, the more will the entire scheme be open to suspicion and the stronger must be the evidence that it does work, that it will in reality enable us to predict results not otherwise predictable. Let us see if this is true of Punnett's three-factor hypothesis. When applied to the F_2 generation from this cross, Punnett's scheme fits the observed results fairly well. But the real test of a scheme built up on so many arbitrary and independent assumptions is to apply it to other crosses than that which it was apparently framed to meet, the peculiar F_2 distribution. If, for example, it fits a back-cross test as well as the F_2 test, it will gain much in acceptability. Punnett makes such an application in his Table II and apparently is fairly well satisfied with the result, better at any rate than with admitting "modification" under any circumstances.

But Punnett's calculated distribution differs from the actual one in the following essential regards. The lower calculated group shows two chief modes instead of the actual one mode. The more pronounced of the two calculated modes differs widely in position from the actual mode. The upper calculated group is far from symmetrical and shows its chief mode on the wrong grade.

It appears therefore that, while Punnett's three-factor hypothesis may, with the aid of a number of carefully framed secondary hypotheses, be made to fit fairly well the observed results for the F_2 generation, it fails to agree at all well with the observed results in the more critical

back-cross. An hypothesis which works only part of the time cannot be said to work well.

Why resort to an hypothesis so complicated when a much simpler one will work better? Punnett's reasons apparently are two, (1) a distrust of explanations involving multiple allelomorphs and (2) a dislike for the idea of "modification" as implying impurity of the gametes. Now multiple allelomorphism, under whatever name one chooses to discuss it, is no longer an hypothesis but a well-established fact. Even in rabbits we have two other undoubted cases, one being the albino series of three allelomorphs (Polish, Himalayan, and coloured), the other the agouti series, likewise of three allelomorphs (grey, black-and-tan, and non-agouti). In guinea-pigs there are four allelomorphs in the albino series (Wright), and three in the extension series (Ibsen). In Drosophila there are eight or more allelomorphs in the white-eye series (Morgan), and we have conclusive proof that the linkage relations with other factors are the same throughout the series showing that all occupy the same "genetic locus." If one chooses to regard a series of allelomorphs as constructed on one and the same genetic basis, with additions or subtractions in individual cases, there can be no valid objection to such a view. Thus Himalayan albinism may be regarded as Polish albinism plus a darkening factor inseparably coupled with it, if one prefers that form of description, but the fact is not altered thereby that in crosses the two things behave as ordinary allelomorphs. So, I maintain, it is with different types of Dutch marking in rabbits. I have identified three such types. Punnett has identified positively one of the same types (White) and tentatively recognizes two other types different from any of mine. He seems to regard my two other types as dubious because they are not the same as his. My experience leads me to be more charitable. I am ready to admit the probable existence of five types of Dutch and that all will probably be found to be allelomorphs. I have even found that a very different type of white spotting which occurs in rabbits, viz. English, behaves as an apparent allelomorph of White Dutch. It may, however, only be very closely linked with Dutch, for it seems remarkable that two genes so manifestly different in their physiological properties should be variants of a single genetic locus. But in any case it is not to be inferred that white spotting in mammals generally consists of allelomorphs of one genetic locus. For Little, Detlefsen and Dunn have independently shown that piebald spotting in mice is genetically distinct from black-eyed-white spotting, the two genes manifesting no linkage (Dunn).

Punnett's second and strongest objection to the hypothesis of multiple allelomorphs seems to lie in what he characterizes as "its necessary concomitant of mutual modification." I trust that this objection will disappear with the explanatory addition which I am quite ready to make that the mutual modification is probably the result of multiple modifying factors which segregate and recombine independently of the chief factor with which they were associated in each uncrossed race. Modification, like the occurrence of multiple allelomorphs, is no longer an hypothesis but a well-established fact. The only point concerning which a reasonable doubt can exist is the correct theoretical explanation of modification. Punnett and I agree in the view that modifying factors are involved in the present case. He thinks that three factors in all will suffice to explain it. I am inclined to invoke a larger number of modifying factors, but identify positively only a single set of allelomorphs of one primary Dutch factor.

IS THE FERTILE TORTOISE-SHELL TOM CAT A MODIFIED FEMALE?

By C. C. LITTLE.

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Doncaster's recent suggestion[1] that the tortoise-shell tom cat might be the result of the process of hormone action during embryonic life, analogous to that producing free martins in cattle, is an interesting one. The whole question of tortoise-shell tom cats has been so frequently discussed, that it is certainly to be hoped that someone with adequate facilities will shortly put this last suggestion to an experimental test. Inasmuch, however, as any breeding work with cats involves considerable time and expense, it seems desirable to point out certain fundamental difficulties which Doncaster's hypothesis meets with at the outset. Sterility in tortoise-shell tom cats, which appears to be the rule when such animals are found, is equally explicable on the hypothesis of "non-disjunction" advanced by the writer and on that of hormone action advanced by Doncaster.

Both hypotheses predicate the formation of sterile males of colours other than tortoise-shell, which would, because of their similar somatic appearance to ordinary fertile males, be easily overlooked. Where there is presumably an XX condition of the sex chromosome in the female, non-disjunction is theoretically possible, and similarly where there are embryos in close juxtaposition within the same horn of the uterus, free martinism is theoretically possible. So far then as the sterile male forms are concerned, both hypotheses have a certain basis for acceptance in observed experimental fact.

The real difficulty comes when a fertile tortoise-shell tom cat is encountered. In this case the hypothesis of non-disjunction is able to account for the observed facts, provided peculiar somatic behaviour of a pair of colour characters already showing an unusual type of coat pattern

[1] *Journal of Genetics*, Vols. VIII. and IX.

is admitted. Furthermore, the breeding behaviour of such fertile tortoise-shell males, can, on the hypothesis of non-disjunction be predicted. When this is done, the prediction coincides with the observed experimental results. The animal should breed like an ordinary yellow male, as the animal reported by Sir Claude Alexander appears to have done.

On the hypothesis of hormone action, the appearance of a fertile male individual representing a completely transformed female is, in itself, a difficult situation to imagine. In addition, no reason is apparent why such an animal should breed as a yellow rather than as a tortoise-shell or as a black. If such an animal was, immediately after fertilisation, an XX (♀) zygote, it is difficult to conceive how, even though its physiological character and sex behaviour were altered to that of an XY (♂) individual, its chromosome content could likewise have been altered. If its chromosome content was not altered, it remained an XX individual. Since, however, it was physiologically male, it could of course, only be mated with *normal* XX individuals (♀ ♀). A mating of XX with XX should have given only XX (♀) offspring. The fertile tortoise-shell male, however, sired both male and female offspring, according to Doncaster's statement[1], 1913, p. 19[2].

Before entering into a series of breeding experiments to determine the existence of non-disjunction in cats, or attempting to collect material for testing the hypothesis of hormone action, it should therefore be taken into consideration that the following situation exists: The hypothesis of non-disjunction, proposed by the writer, while admittedly more difficult of experimental proof than that described by Doncaster, involves no fundamental changes in the chromosome mechanism of sex determination. Its most visionary phase involves continued irregular somatic behaviour of coat colours already shown to be peculiar in this respect in the tortoise-shell female cat.

[1] *Journal of Genetics*, Vol. III.

[2] There is some question even in the case of the one surely fertile male reported, but had his progeny all been of one sex, the fact could scarcely have escaped comment, since he produced many offspring.

THE GENETICS OF A *DAPHNIA* HYBRID DURING PARTHENOGENESIS.

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(With Three text-figures.)

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Introduction.

On the hypothesis, now supported by such a mass of indirect evidence, that segregation of hereditary factors is due to the segregation of homologous chromosomes in meiosis, it is clear that there should be no such segregation in parthenogenetic reproduction unaccompanied by reduction of the chromosomes. The determination of whether or not segregation does occur under these circumstances may indeed be considered a crucial test of the truth of the hypothesis. At present there exists very little direct evidence on this point, at any rate for the animal kingdom.

For many years I have been conducting experiments with Cladocera, in which both males and females are produced parthenogenetically without reduction of chromosomes, with the purpose of solving this problem for this group of animals. It is however only recently that I have succeeded in obtaining the hybrids necessary for its direct solution, though indirect evidence of the absence of segregation in parthenogenesis in this group was presented in my paper of 1914.

Some Points in the Biology of the Cladocera which concern the present experiments.

The Cladocera reproduce both sexually and parthenogenetically. In the former type of reproduction the egg forms two polar bodies in the usual way. The fertilized egg always, so far as is known, develops into a female. This female produces young parthenogenetically, and these, if females, may do so again. Parthenogenetic reproduction may thus be continued for a very large number of generations, and probably indefinitely. One of my own lines of *Daphnia obtusa* has been bred in this way for over a hundred generations and is still in as strong condition as at first. Sooner or later, however, sexual reproduction makes its appearance by the production of males and of eggs requiring fertilization. The males are produced parthenogenetically like the females, and, like these, without reduction of chromosomes (Chambers, Taylor). The appearance of sexual forms does not bring parthenogenesis to an end, but the two methods continue side by side. Thus, for example, a female may produce parthenogenetically a brood of females, then a couple of eggs requiring fertilization (referred to hereafter as sexual eggs), then returning to parthenogenesis it may produce a brood or two of males, and then a few broods of females. In most of the lines of *Daphnia* and *Simocephalus* which I have bred, parthenogenetic production of females has been predominant, only a small percentage of males and sexual eggs appearing now and then.

As a rule, all the members of one parthenogenetic brood (at any rate in *Daphnia*) are of the same sex, but mixed broods are not uncommon.

The part played by the environment in determining sexual or parthenogenetic reproduction in these animals has frequently been made the subject of experiment. This side of the question does not concern us here, and it must suffice to say that it has been satisfactorily established that, at any rate in the species with which we are dealing here, there is no hard and fast predetermined reproductive cycle independent of environment.

Parthenogenetic eggs, whether destined to develop into males or females, pass out of the oviduct into the brood pouch where they undergo their development, escaping to the exterior four or five days after laying (at room temperature). The number of parthenogenetic eggs laid may vary from one to thirty or more, the number depending mainly on the abundance of the food supply. Sexual eggs are however always produced

(in *Daphnia*) two at a time, one from each ovary. When ripe they are likewise passed out of the oviducts into the brood pouch.

Even when the eggs are at a very early stage of ovarian growth, it is easy to determine through the transparent tissues of the animal whether they are sexual or parthenogenetic. The two kinds of eggs differ in colour and texture; moreover, in the single sexual egg in each ovary yolk deposition starts at the hind end and thence spreads forwards, while in a parthenogenetic ovary yolk appears simultaneously in a number of eggs spaced out along the length of the ovary.

Copulation and fertilization in the Cladocera was long ago described by Weismann (1880), and I can confirm his account in most particulars. Briefly, if a female with sexual eggs in her ovaries is kept from copulation, the eggs are not laid, but are retained in the ovaries till they disintegrate. If copulation takes place, the eggs are laid into the brood pouch, where they meet with the spermatozoa. If fertilized they become enclosed in a special egg-case or "ephippium" secreted by the brood pouch; if not fertilized, they disintegrate in the brood pouch within a few hours.

During copulation the male introduces the tip of his abdomen into the female brood pouch, and the spermatozoa are passed into it. Generally within a very few minutes after copulation the two eggs are laid into the brood pouch. The stimulus of copulation seems to be almost essential if oviposition is to take place. In the course of these experiments I have had occasion to observe this particularly in the case of more than 400 pairs of sexual eggs produced in the absence of males. In only four of these cases were the eggs laid—in each case of course disintegrating soon afterwards. Further proof that it is copulation which induces oviposition is furnished by matings in which the male is a sterile hybrid (to be described below). In all such cases the eggs were laid shortly after copulation, though as the males contained no spermatozoa the eggs could not be fertilized and disintegrated soon after laying.

Eggs that have been laid after normal copulation meet with the spermatozoa in the brood pouch and are fertilized. At first the eggs are irregular in shape, but they soon round themselves off and take up a position one behind the other in the middle axis of the body. The walls of the brood pouch secrete the thick chitinous ephippium which encloses the eggs and is cast off at the next ecdysis, which takes place about 3—4 days after oviposition. The ephippium, containing its two eggs, lies at the bottom of the water for an indefinite period. The contained eggs may hatch in a few weeks, or not till after several years.

An ephippium is always formed when sexual eggs are produced in the ovaries, whether these eggs are laid or not. In the latter case the ephippium is cast empty after the usual interval has elapsed since the time at which oviposition should have taken place.

A feature of great importance for an experiment in which bodily dimensions are concerned is that the Cladocera, like so many other Arthropods, only increase in size at each ecdysis. Between two ecdyses their dimensions do not undergo any change detectable by the micrometer. The ecdyses and mode of growth etc. of the Cladocera was described in some detail in my former papers (especially 1913), and need not be redescribed here. During the interval between the last ecdysis before the first batch of eggs is laid and the ecdysis which immediately precedes the first oviposition, the animal is said to be in the *adolescent instar* or stage. Between the last mentioned ecdysis and that which immediately precedes the laying of the second batch of eggs, the animal is in the *first adult instar* or stage. Then follows the second adult instar, and so on.

Culture methods.

The methods used for culture were in the main the same as those employed before and described in my previous papers. For food however I used a culture of green flagellates—mainly *Euglena* and Chlamydomonads—grown in a solution made by boiling 25 gms. of canary guano in 11 litres of water and afterwards filtering. These cultures were kept in large battery jars and provided an inexhaustible supply of food. After a few months, the bottoms of these culture jars became carpeted by encysted organisms to a depth of several millimetres.

Another departure from the methods previously described by me was that, instead of isolating each individual at birth, up to about six members of each brood, verified to be of the same sex, were generally placed immediately after birth in one tumbler and kept there till maturity, when they were isolated into separate $4'' \times 1''$ breeding tubes.

The experimental data.

Material and Characters employed.

My first experiments (1908) were attempts at intergeneric crosses between *Daphnia* and *Simocephalus*, but no fertilized eggs were obtained. Indeed, no attempt at copulation was ever observed. The experiments now to be described concern species crosses within the genus *Daphnia*.

Species of *Daphnia* are mostly differentiated from one another by small quantitative characters which are difficult to define precisely, though no one who has brought into cultivation numbers of clones of this genus could doubt for a moment that an enormous amount of genetic diversity exists within the genus, and indeed within each species, or even each named variety (cf. Agar, 1914).

The eleven clones with which this experiment was started can easily be divided into two groups, one referable to *D. pulex* and the other to *D. obtusa*. The females of these two species are very similar in regard to their morphological characteristics. Indeed, the only even fairly satisfactory distinguishing feature, capable of precise definition, which I could find, is the relative sizes of the three dorsal abdominal processes. In the females, the differences in these processes are not very great, and transgressive fluctuation takes place. In the males however the middle of the three processes is six or seven times as long in *D. pulex* as in *D. obtusa*, although the body length of the male *D. pulex* is only greater than that of *D. obtusa* in the proportion of about 5 : 4. Thus it is never possible to confuse adult males of these two species.

The relations of these abdominal processes are shown in Figs. 1 and 2 (pp. 6, 7).

In the female, process *C* has obviously the function of closing the brood pouch posteriorly and so of preventing the premature falling out of the eggs and embryos. When the moment arrives for the young to be born, the mother flexes her abdomen ventrally, thus withdrawing the process from the brood pouch, and leaving a way of escape for the young. Processes *B* and *A* may possibly help indirectly in closing the brood pouch by strengthening process *C*.

As the figures show, the same three processes are present in the male, but only *B* is well developed. It does not seem possible to ascribe any function to this single well developed process. It is probably an example of the appearance in one sex of an organ which is only functional in the other. Its hypertrophy in *D. pulex* as compared with *D. obtusa* also points to the conclusion that it is a functionless organ.

Owing to the great influence of environmental differences, especially of food and temperature, on the size of these animals, the absolute lengths of these abdominal processes would be unsatisfactory to use. Consequently a ratio was always employed which eliminated the greater part of this environmental influence. The ratios in question are in the female: (1) the ratio between length of body and length of process *B* $\left(\frac{L}{B}\text{ ratio}\right)$, and (2) the ratio between the lengths of processes *B* and *A*

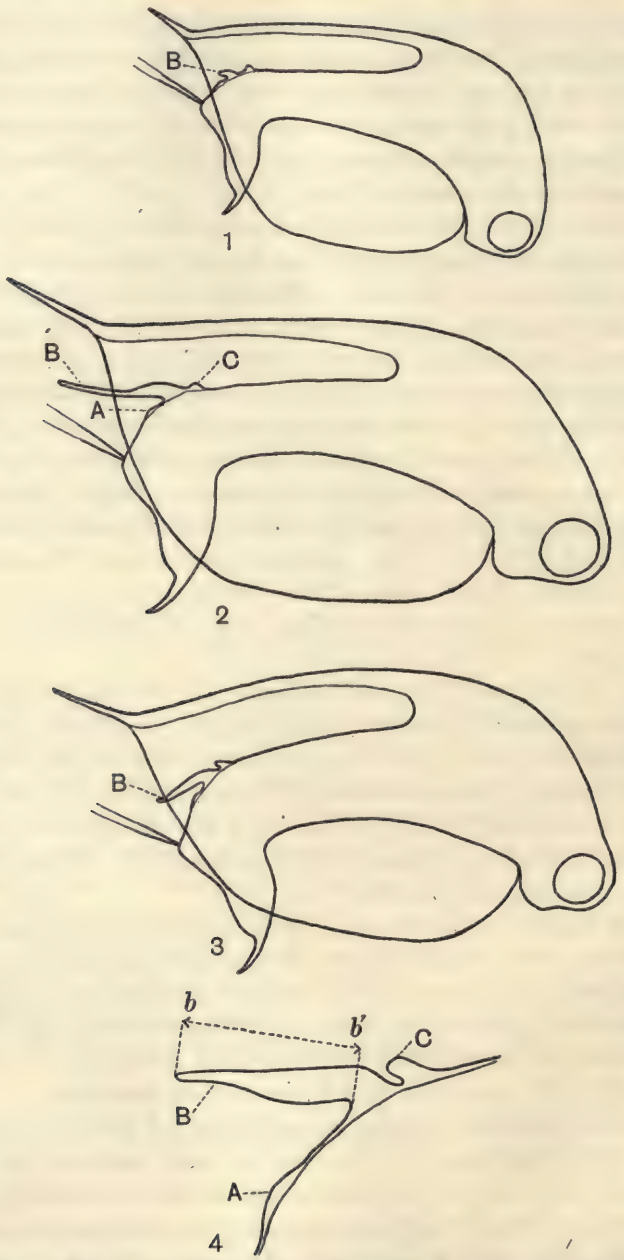


Fig. 1. Males of *Daphnia*. (1) *D. obtusa*; (2) *D. pulex*; (3) hybrid, *D. obtusa* × *D. pulex*; (4) dorsal abdominal processes of a hybrid on a larger scale; *A*, *B*, *C*, the three dorsal abdominal processes; *b*—*b'*, the line along which the measurements of process *B* were made.

$\left(\frac{B}{A}\text{ ratio}\right)$. The latter ratio was found to be the more useful. In the male the ratio $\frac{L}{B}$, as in the female, was employed. In both sexes, length of body was taken as a straight line from front of head to base of spine (Fig. 2).

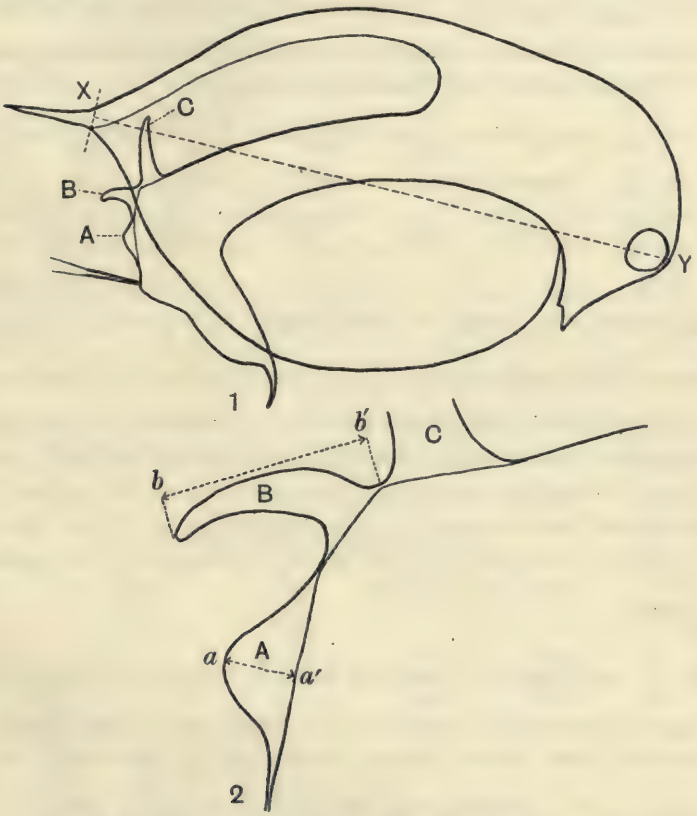


Fig. 2. Female *D. pulex*. *A*, *B*, *C*, the three dorsal abdominal processes; *a*—*a'*, *b*—*b'*, the lines along which the measurements of processes *A* and *B* were made; *X*—*Y*, the line along which the body length was measured.

Like most other sexual characteristics, the abdominal processes do not attain their full development until maturity. The new-born female has no recognisable trace of process *C*, and only slight sinuosities of the dorsal outline to represent *A* and *B*. By the time the adolescent stage is reached, all three processes are well developed, but still much smaller than in the adult, not only absolutely, but also relatively to the body

length. The length of the processes continues to increase relatively to the length of the animal even after the adult stage is reached, so that the $\frac{L}{B}$ ratio decreases with successive ecdyses. Thus in nine females of *D. obtusa* (clone *H*), measured both in first and second adult stages, the average $\frac{L}{B}$ ratio in the first stage was 17·1 and in the second 14·4. Four females of the same species measured both in adolescent and first four adult stages gave the following mean figures:

Ratio	Adolescent stage	First Adult stage	Second Adult stage	Third Adult stage	Fourth Adult stage
$\frac{L}{B}$	19·6	17·4	15·2	14·1	13·1
$\frac{B}{A}$	1·85	1·91	2·23	2·20	2·22

It is thus clear that it is imperative that all animals should be measured at the same stage of development, and the stage chosen was the second adult stage. Even with this precaution, the $\frac{L}{B}$ ratio has the disadvantage that larger animals tend to have lower ratios than smaller ones even of the same instar, showing that large animals have relatively even larger abdominal processes. Since the size of the animal is very greatly affected by changes of food, temperature, etc., these factors also tend to obscure the genetic constitution of the animals as expressed in the $\frac{L}{B}$ ratio.

The $\frac{B}{A}$ ratio, however, does not suffer so much from this drawback. Although larger animals tend to have larger ratios than smaller ones of the same instar, the difference is not great. Consequently this ratio is less affected by environmental differences than the $\frac{L}{B}$ ratio, and therefore has been the characteristic chiefly used (in the female). As we shall see, however, even this feature is sufficiently sensitive to environment to obscure genetic composition to some extent.

It will also be noticed from the above figures that the $\frac{B}{A}$ ratio reaches approximate equilibrium in the second adult stage, which, it will be remembered, is the stage in which all females were measured.

As in the females, the abdominal processes of the males are relatively very small at birth, and so it is necessary here also to make measurements

as nearly as possible at the same stage of the life cycle. The successive adult stages of the female are conveniently marked off by the laying of a batch of eggs immediately after each ecdysis. In the male there are no such prominent landmarks, and in order to insure that all specimens measured should be as nearly as possible at the same stage of growth, the males were measured when 20—21 days old—that is to say, about twelve days after attaining sexual maturity. Even at this age, however, there is a good deal of variation in body length caused by environmental influence, and as on the whole large males have relatively even larger abdominal processes, the $\frac{L}{B}$ ratio in the male is subject to the disturbing effects of environment as in the female. Fortunately, the difference between the male ratios in the parent species is so much greater than the modification caused by environment that the latter do not cause any serious inconvenience.

In order to make the measurements, the animals were anaesthetised by placing for a minute or two in 1 °/₀ ether solution[1]. They were then placed on a slide and measured with an eye-piece micrometer under a combination of lenses which would give a measurement extending over as long a stretch of the scale as possible. The values of the units of

TABLE I.

Mean ratios of various clones.

Ratio	Clone	Number of specimens measured	Mean
♂ $\frac{L}{B}$	*D. obtusa*		
	Clone *H*	4	30·8
	,, *M*	48	37·3
	,, *K*	20	25·6
	D. pulex		
	Clone *RR*	28	6·2
♀ $\frac{L}{B}$	*D. obtusa*		
	Clone *H*	67	14·7
	,, *M*	44	15·2
	D. pulex		
	Clone *RR*	33	14·2
♀ $\frac{B}{A}$	*D. obtusa*		
	Clone *H*	75	2·17
	,, *M*	44	2·10
	D. pulex		
	Clone *RR*	69	2·89

[1] This causes no ill effects. The animals may be kept completely anaesthetised in this strength of ether for several hours, and recover perfectly on return to pure water.

measurement were: in the case of the ♀ body length ·0371 mm., ♂ body length ·0309 mm., and abdominal processes ·0062 mm. Before finding the ratio between body length and abdominal process, the former had therefore to be multiplied by 5·99 (counted as 6) in the ♀, and by 4·99 (counted as 5) in the ♂. The lines along which the measurements were taken are shown in Figs. 1 and 2.

The mean values for these ratios in the clones chiefly employed are shown in Table I.

It will be noticed that the ratio $\frac{L}{B}$ in the female differs but little in the two species. As in addition it has the drawback of being powerfully affected by the same features of the environment as influence the general size of the animal, no further use is made of this ratio in the ♀ in these experiments.

The production of the hybrids.

In 1914, acting on experience acquired in the breeding of numerous clones of Daphnids, I obtained specimens of *Daphnia* from a number of different localities, and worked up a clone from a single parthenogenetic female from each locality. All the clones employed were referable to the two species[1], *Daphnia obtusa* (8 clones) and *D. pulex* (3 clones). Cross matings were carried out between the two species, as well as control matings within the species (both between clones, and between members of the same clone). Every individual was examined at birth, and the few males isolated from the females. In order to make a cross, an adult female with young sexual eggs in her ovaries was placed with a male until the eggs were laid into the brood pouch, after which the male was removed for use with another female. The fertility of these matings can be summarised as follows (Table II):

TABLE II.

Fertility of Matings.

Type of mating	Number of successful matings	Number of unsuccessful matings	Percentage of successful matings
A. *D. obtusa* × *D. obtusa*			
1. intraclonal ...	41	8	84
2. interclonal ...	40	12	77
B. *D. pulex* × *D. pulex* intraclonal ...	24	1	96
C. *D. obtusa* × *D. pulex*	38	97	28
D. *D. pulex* × *D. obtusa*	12	55	18

[1] I am indebted to Mr D. J. Scourfield for kindly confirming my identification of these two species.

In the above table, an "unsuccessful mating" is one in which the eggs were not laid, or if laid, disintegrated in the brood pouch, showing that they had not been fertilized. If the eggs became rounded off and took up their proper position in the ephippium without disintegrating in the few days which elapses between oviposition and the casting of the ephippium, it was assumed that they had been fertilized, and the mating is recorded as successful though only in a few cases was this confirmed by observation of the hatching of the egg. Cases where only one of the two eggs was fertilized and the other disintegrated are counted among the successful matings.

In compiling the above table, all those failures have been omitted in which there was good reason to believe that the male was either too old or too young, or in which the female had been placed with the male less than 24 hours before the ephippium was cast, since it was found that if copulation were delayed till that time the eggs were rarely laid, even in straight matings. The optimum time for copulation to take place, and therefore for the eggs to be laid, appears to be 3—4 days before the ephippium is ready to be cast. At this time it is still very young and soft; later it becomes rigid and possibly this may oppose a mechanical obstacle to effective copulation or to oviposition.

A glance at the above table will show that the percentage of successful interspecific matings is significantly less than in the case of matings between members of the same species. The difference between the percentages for the inter- and intra-clonal matings in *D. obtusa* is probably within the limits of experimental error, and therefore no significance should be attached to it.

The first series of crosses were carried out in the spring and early summer of 1914, and resulted in 29 egg-containing ephippia from crosses between the two species. None of these, however, had hatched by the autumn of that year, when circumstances prevented the continuation of the experiment. Some of these ephippia were left in a dry condition, and some in water, and they were all re-examined on Dec. 31, 1918. All the eggs in the dried ephippia had disappeared, but several of the ephippia which had been in the water still contained apparently good eggs. On June 25, 1919, one egg hatched from a mating between two clones of *D. pulex* made on June 13, 1914. This egg had therefore been lying dormant in water for five years. So far as I know, there was no change in the conditions to which one could assign the stimulus which caused it suddenly to resume development after such a long dormant

period. The ephippium was in a small tube containing only two or three cubic centimetres of water, standing in my laboratory. Unfortunately, this animal died when about a week old, owing to fungoid growth.

The hybrids to be described below were all derived from a fresh set of crosses carried out in the year 1919. Some 21 fertile ephippia from crosses between the same two species, and 22 from straight fertilizations were obtained in the early part of that year, and one or both of the eggs in eight of these had hatched by May, giving eleven young. The interval which elapsed between fertilization and hatching in these eggs varied from six to sixteen weeks. The young hatching from these ephippial eggs experienced a very high mortality, no less than seven dying before their first ecdysis, and one other as a young adult. A summary of these eleven young and their fates is given below.

Type of mating	Number of young	Fate
D. obtusa × *D. obtusa*	1	died as young adult
D. pulex × *D. pulex*	7	6 died young, 1 gave rise to clone *RR*
D. obtusa × *D. pulex*	2	1 died young, 1 gave rise to clone *Xc*
D. pulex × *D. obtusa*	1	gave rise to clone *Xf*

By far the greater part of the work was done with the hybrid clone *Xc*, and this we will now describe.

Genetics of the hybrid clone Xc.

The origin of this clone was, then, the mating *D. obtusa* (clone *H*) × *D. pulex* (clone *R*). On attaining maturity, the hybrid ♀ immediately showed one of the most striking characteristics of the hybrid clone to which it gave rise, namely a marked derangement of its reproductive processes. Thus the history of its first three broods is as follows:

March 30. Six parthenogenetic eggs in brood pouch.
April 2. Five of the eggs broken up, one has developed into an embryo.
April 5. This embryo (a ♂) born. Parent has four more parthenogenetic eggs in the brood pouch.
April 7. Two of these eggs broken up.
April 9. One living young only born (a ♂). Parent has ten more eggs in the brood pouch.
April 10. All eggs dead except one.
April 11. One ♀ born. Parent has fourteen eggs in brood pouch (4th brood).

After this she produced a number of broods (some ♂ and some ♀) more successfully, with only a small proportion of eggs failing to develop.

The two peculiarities of this history are of course the early production of ♂'s and the large proportion of eggs which failed to develop. A third peculiarity made its appearance among the daughters of the hybrid—

namely, excessive production of sexual eggs, and these three features continued to manifest themselves throughout the ten generations for which the clone has been bred. Some females showed indeed a much greater degree of sterility than the original hybrid. One ♀ in generation 3, for example, started by producing two or three sets of sexual eggs, then seven successive batches of parthenogenetic eggs broke up in the brood pouch without undergoing any visible development. The eighth parthenogenetic brood consisted of three eggs, of which two failed to develop and one died as a young embryo. Two more sets of parthenogenetic eggs were then laid, all of which disintegrated without development. The parent then died, having only produced one dead embryo out of ten batches of parthenogenetic eggs.

Several other females of this clone laid batches of parthenogenetic eggs regularly, but never produced any living young.

These are of course extreme instances, but a high degree of partial sterility was general in the females of this hybrid clone. It is difficult to give figures of the proportion of eggs which failed to develop, since the majority of them break up very soon after laying, and unless one happens to observe the ♀ immediately after oviposition, nothing remains of them but loose grains of yolk. There is no doubt however that considerably more than half the parthenogenetic eggs produced by this clone have failed to develop.

A certain amount of pre-natal mortality has been evident even in my pure bred *Daphnia* clones, especially of *D. pulex*, but the rate does not approach that of the hybrid clone.

Records were taken of the sexes of the living broods produced by eighteen of the daughters of the original hybrid, with the following results:

Nature of Brood	Number of Broods
A. Sexual eggs	37
B. Parthenogenetic eggs:	
♂ broods...	24
♀ broods...	24
Mixed ♂ and ♀ broods...	6

I have no exact records of the properties of the different types of broods produced by the two parental clones, but from a long experience of them I should estimate that *D. obtusa*, clone *H*, produces not more than 3 °/₀ of sexual broods and 1 °/₀ of male broods, and *D. pulex*, clone *RR*, not more than 10 °/₀ sexual broods and 5 °/₀ male broods. These figures apply of course to the conditions under which they and the hybrid clone were bred.

It is curious, in view of the imperfect fertility of the parthenogenetic eggs, and of the complete sterility of the hybrid males, to be described below, that the sexual eggs produced by the hybrid females seem to be perfectly fertile when these are mated to males of the two parental species. These matings give about the same percentage of fertilized eggs as matings between pure bred members of the same species. This is shown by Table III, which should be compared with Table II. The criterion for deciding whether an egg has been fertilized is the same as in Table II, namely the fact that it becomes rounded off and takes up its proper position in the ephippium. Up to the time of writing, none of these eggs had hatched.

TABLE III.

Fertility of Matings with Hybrid Females.

Type of mating	Number of successful matings	Number of unsuccessful matings	Percentage of successful matings
Hybrid ♀ × *D. obtusa* ♂ ...	44	8	85
Hybrid ♀ × *D. pulex* ♂ ...	24	6	80

Finally, the hybrid clone exhibited the important feature of complete sterility of the males. This is a phenomenon which has often been recorded in interspecific crosses, even where the females are fertile; for references the reader is referred to Detlefsen.

In the normal fertile male of the parent species, the testes are easily seen through the transparent tissues of the living animal, and when mature the refractive spermatozoa can be seen floating in a fluid which flows up and down in the tubular testis with the motions of the animal's abdomen. The testes of the living hybrids present quite a different appearance. Though of the same size and distinctness, they appear solid, and no spermatozoa are visible. Many males, belonging to different generations of the hybrid clone, were sectioned, and the absence of spermatozoa was confirmed by microscopic examination. The spermatogenesis of these hybrids is being worked out by another investigator, and at present it must suffice to point out that the full size of the hybrid testis, combined with the absence of spermatozoa, indicates that spermatogonial mitosis proceeds successfully, but that the meiotic phase is not completed.

Nine males were tested with females. They copulated freely, with the result that the eggs were laid, but all disintegrated soon after, showing that they had not been fertilized.

Summing up, the clone, or group of individuals obtained parthenogenetically from the hybrid female *Xc*, showed the following features in

TABLE IV. *Pedigree chart of the clone descended from the hybrid female Xc. Only the individuals which were measured are shown.*

Generation																		
1									♀ = *Xc*									
2	1♀	1♀	1♀	1♀	1♀	1♀	1♀	1♀	1♀	1♀	1♀	1♀	1♀	1♀	1♀	1♀	1♀	27♂
3	3♀ 15♂	10♀ 7♂	3♀	4♀ 4♂	4♀	1♀ 6♂	2♀ 13♂	5♀ 11♂	3♀ 7♂	5♀ 11♂	3♀	5♀ 7♂	6♀ 3♂	6♀ 6♂	5♀ 4♂	4♂	6♀ 7♂	
4	2♂	10♀ 6♂	10♀ 4♂	6♀	5♀		4♀	5♀	2♀ 7♂	2♀	5♀	6♀ 6♂	10♀	5♀ 6♂	5♀		2♀	
5		3♀	3♀ 5♂	2♀	1♀		6♀ 2♂	2♀	3♀	2♀	2♀	1♀	2♀	2♀	2♀			
6		2♀	4♀	2♀	2♀		2♀	2♀		2♀	2♀	4♀	2♀	2♀				
7		2♀	2♀		2♀		2♀	1♀		6♀ 6♂		1♀ 5♂	6♀ 1♂					
8		4♀ 1♂	8♀ 4♂		2♀		2♀	1♂		9♀ 13♂			7♀ 4♂					
9		6♀ 10♂	6♀ 53♂				10♀ 12♂			2♂								
10		1♂					6♂											

regard to its reproductive processes, and these features were maintained throughout the ten generations during which it has been observed.

(1) Excessive production of sexual eggs.
(2) Excessive production of males.
(3) Complete sterility of the males.
(4) Imperfect fertility of the parthenogenetic eggs.
(5) Probably normal fertility of the sexual eggs.

Ten generations of the hybrid clone have been bred for this experiment, counting the original hybrid ♀ as generation 1. Seventeen daughters of this ♀ were used each to found a line, though owing to the high degree of sterility, a number of lines died out in the first few generations. A condensed summary of the whole hybrid population is shown in the pedigree chart constituting Table IV. It will be understood of course that in the original records the individual offspring of individual parents were recorded, but in the chart only the total number of males and females measured in each line in each generation are shown.

The means for the $\frac{L}{B}$ ratio of the ♂'s, and of the $\frac{B}{A}$ ratio of the ♀'s, of the hybrid clone and of the two parent forms are given in Table V. The male parent of the hybrid belonged to clone *R* of *D. pulex*. Very few measurements were made of members of this clone, which was unfortunately allowed to die out. Instead, the figures are given for clone *RR*, which was descended from a female produced sexually by a straight fertilization of a clone *R*♀ by a clone *R*♂. This clone can therefore be considered, for all practical purposes, identical with clone *R*.

TABLE V.

Mean Ratios for the Hybrid and Parental Clones.

	$\frac{L}{B}$ males		$\frac{B}{A}$ females	
	Number of males measured	Mean	Number of females measured	Mean
D. obtusa, clone *H* ...	4	30·8	75	2·17
D. pulex, clone *RR* ...	28	6·2	69	2·89
Hybrid clone	281	10·1	226	1·97

Inheritance within the hybrid clone Xc.

A glance at the results of these experiments is enough to show that segregation of a single pair, or even of only two or three pairs, of factors is not taking place. It is however common in species crosses for any given character difference between the two parents to depend upon a large

number of factorial differences, and hence more refined methods for detecting segregation are required. In such cases, the pure parental combinations can only be expected to appear very rarely among large numbers of descendants of the hybrid, and segregation is most readily detected by an increase in the range and amount of variation in F_2 and subsequent generations as compared with F_1. If the parents are homozygous, any diversity in F_1 depends upon purely non-genetic factors acting on genetically homogeneous material, while in the later generations, though the non-genetic causes of diversity remain the same, the material is now genetically heterogeneous, and hence the total amount of diversity is increased. If the number of factors is so large that all the possible combinations are only likely to appear when the total number of individuals is very great, the range of variation in the population found by the descendants of the hybrid will continue to increase until the extreme combinations have made their appearance. Moreover, while any individual or pair of individuals from the F_1 generation should give offspring not differing, except within the limits of error of random sampling, from those of any other F_1 individuals, the groups of offspring of different individuals of the F_2 generation will differ from each other. In general, F_2 parents will produce F_3 groups into a mean near to that part of the range of variation to which they themselves belong. In other words, there should be a correlation between parent and offspring in all generations later than F_1.

Examples of this method of detecting segregation among the factors co-operating to give the distinctive features to complex somatic characters are now among the common places of genetics, and the same methods can be applied to a hybrid reproducing itself parthenogenetically. Indeed, segregation must be much more easily detected in such a case, since the results of segregation cannot be neutralized by recombination of the segregated factors in syngamy.

The material yielded by the clone *Xc* has been tested in both these ways. The test of variability, as measured by the standard deviation and range of variation has been applied to the males, both because this is the simplest test, and because their sterility excludes the direct application of the correlation test. This test has however been applied to the females.

The relevant data regarding the inheritance of the $\frac{L}{B}$ ratio in the males of the hybrid clone are summarised in Table VI, which also

TABLE VI.

Distribution of $\frac{L}{B}$ *ratio in males of* D. obtusa, *clone H*, D. pulex, *clone RR, and of the different generations of the hybrid clone Xc.*

In order to compress the table, class intervals are twice as large as those used elsewhere in this paper. For explanation of letters *a* and *b*, see text.

Clone	Ratio 4·5	5·5	6·5	7·5	8·5	9·5	10·5	11·5	12·5	13·5	14·5	15·5	16·5	17·5	18·5	19·5	20·5	21·5	22·5	23·5	24·5	25·5	26·5	27·5	28·5	29·5	30·5	31·5	32·5	33·5	34·5	35·5	36·5	37·5	Number measured	Mean	σ
H	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	1	–	–	–	–	–	–	1	4	30·80	–
RR	5	10	7	3	2	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	28	6·21	1·433 ± ·129
Xc, Generation 2	–	–	–	–	4	9	4	5	2	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	26	10·42	1·581 ± ·148
Xc, Generation 3	–	–	–	–	2	47	43	9	2	–	–	–	–	–	–	–	–	–	–	–	–	*a*	–	–	–	–	–	–	–	–	–	–	–	–	103	10·13	·728 ± ·033
Xc, Generation 4	–	–	–	1	5	5	14	5	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	31	10·20	1·334 ± ·114
Xc, Generation 5	–	–	–	–	–	5	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	9·89	–
Xc, Generation 7	–	–	–	–	5	2	3	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12	9·72	–
Xc, Generation 8	–	–	–	–	2	8	6	5	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	23	10·45	1·120 ± ·111
Xc, Generation 9	–	–	–	1	11	30	18	8	3	2	–	–	*b*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	73	10·03	1·123 ± ·063
Xc, Generation 10	–	–	–	–	–	1	5	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	10·97	–

shows the distribution of the values in the two parental species. The number of males measured in clone *H* of *D. obtusa* is only four, but many more were obtained at other periods of the experiment, only not measured under the conditions necessary for inclusion in this table. Their general characters confirm the values given for this small sample. Males were more abundant in other clones of *D. obtusa*, the values of their mean ratios being given in Table I. The apparent high degree of variability among these four clone *H* males is not very significant. It is plain that when we are dealing with fractions, such as the $\frac{L}{B}$ ratio, of which the numerators are not very different, but the denominators many times larger in one case (*D. pulex* and the hybrid) than in the other (*D. obtusa*), small variations in the small denominators—whether biological or due to experimental error—will produce much larger differences in ratios than variations of equal extent in the larger denominators.

There is no evidence of progressive increase in diversity in successive generations of the hybrid males, whether measured by the standard deviation or by the extreme range of variation. The numbers in each generation are indeed in most cases too small for a satisfactory determination of the actual value of the standard deviation, but it is plain that it is not increasing progressively. If, in order to obtain larger numbers, generations are amalgamated into two groups, one consisting of the earlier, and the other of the later, generations, we get the following result (Table VII):

TABLE VII.

$\frac{L}{B}$ ratios for males of the earlier and of the later generations of the hybrid clone.

Generations	Number	Mean	σ
2+3+4	160	10·19 ± ·06	1·050 ± ·040
7+8+9	108	10·08 ± ·07	1·137 ± ·051

The difference between the standard deviations of the two groups is only ·087 ± ·065, which is plainly not significant.

Before leaving this table, it is necessary to draw attention to certain omissions which were made in its compilation. Altogether nine males, all belonging to the hybrid clone, have been left out of the table for the following reasons.

One complete brood of five males, and one of another brood, giving six in all, when measured at the age of 21 days, were found to be still exhibiting juvenile characteristics, especially of the first antennae, thus indicating that for some reason sexual maturity had been delayed in

these animals. Since, as described above, the relative length of the abdominal processes does not attain its final value till after maturity, these animals were rejected.

One other individual (in generation 3) was rejected because its abdominal process was bent and deformed, so that the value of the ratio (12·75) obtained by measuring the process along the line shown in Fig. 1 could not properly be compared with those of normal specimens.

The two remaining individuals which were omitted were one specimen in generation 3 with a ratio of 23·75 (marked *a* in Table VI) and one in generation 9 with a ratio of 16·75 (*b* in the Table). These individuals—at any rate the first one—obviously fall outside of the normal distribution of the population and could not be properly employed in calculating the statistical constants. They are probably of no genetic significance. They were both very small specimens—indeed they were the smallest $Xc\,♂$'s measured, with the exception of one other which was of the same size as these two. As we have seen, even after sexual maturity is attained, the value of the $\frac{L}{B}$ ratio continues to sink with increase in age, and hence size, of the animals, and hence there is an organic correlation between small size and high ratio.

It will be noticed that it is a general characteristic of the distributions—not only of the hybrid clone, but of the pure species as well—to be more or less markedly skew; the range of variation generally stretches out further on the high than on the low side of the mean or mode. This is doubtless an expression of the fact that favourable conditions produce large animals and hence low ratios. Now favourable conditions imply a combination of environmental factors each differing but little from its optimum condition, and hence variations in the environmental complex towards the side of extreme unfavourableness are more likely to occur than variations towards the other extreme of the range. Hence the skewness of the distribution of the ratio values is probably simply an expression of the skew distribution of the degrees of favourableness of the environmental complex, and I think there is little doubt these two specimens were merely extreme examples of environmental modification.

Another argument against the view that these two specimens are the result of segregation is that the males of clone *H* (which these specimens approach) are as regards bodily size all smaller either than any *RR* male, or than the mean of the *Xc* males. The two aberrant males under discussion, though not reaching the high ratio of the clone *H* males, were

actually smaller in body length than any clone *H* male measured—a confirmation that their high ratios were due to a certain juvenility of character rather than to an approach to the genetic constitution of the *D. obtusa* males. It is also significant that there are no outstanding variations towards the low ratio characteristic of the *D. pulex* parent.

Finally—and most important of all—the testes of the specimen of ratio 23·75 was examined and found to be of the usual hybrid type, containing no spermatozoa. Unfortunately, I have no record of the condition of the testes of the other aberrant male, since it died a few days after measurement, before I had time to make the examination.

It should be mentioned that both these aberrant males belonged to otherwise normal broods. The ♂ of ratio 23·75 had five normal brothers, the highest ratio among them being 10·25; the ♂ of ratio 16·75 had two brothers, of ratios 9·25 and 9·75. Thus if the high ratios of these two males were due to segregation, this cannot have taken place earlier in their ancestry than in the formation of the egg from which each developed.

Taking all these considerations into account—and to them may perhaps be added the judgment formed by many years' experience of the type of somatic modifications caused by environment in Cladocera—I think conclusion unavoidable that two males of abnormally small size and high ratios were not exceptions to the general rule of absence of segregation obtaining in the rest of the clone, but merely examples of extreme somatic modification.

The conclusion to be drawn from this examination of the hybrid males is that no segregation of the factors which determine the $\frac{L}{B}$ ratio has taken place during the course of this experiment. For ten parthenogenetic generations (eight acts of reproduction) all males—and hence all their female ancestors—have remained of the same heterozygous composition as that of the original F_1 hybrid.

The persistence of the characteristic of sterility in the males throughout the successive generations of the hybrid clone is also of course strong evidence against the occurrence of segregation.

As we have seen, there were altogether ten generations of the hybrid clone, counting the original hybrid female as generation 1, and 17 of the female offspring from this hybrid were each used to found a line (Table IV). In constructing the table to test the parental correlation

among the females of the hybrid clone, the original hybrid female was omitted, and the correlation between mother and daughter for the $\frac{B}{A}$ ratio was calculated for the population formed by the 16 of the 17 lines which left female offspring. All generations are combined in the table, and therefore many specimens are counted twice, once as offspring of the nth generation, and again as parents of the $(n+1)th$ generation. Whenever a parent had more than one offspring, it is counted over again with each one, in the usual way. Thus the number of offspring available for the correlation was 276[1], but the actual number of parents is only 77.

The crude correlation table yields a positive correlation coefficient of $\cdot 132 \pm \cdot 039$. This, being more than three times its P.E. must be considered as probably significant. This is, of course, far from equivalent to saying that it is due to genetic resemblance between mother and daughter. Indeed, detailed examination shows that it is almost certainly to be ascribed to environmental causes.

The readiness with which somatic modifications are induced in Cladocera by environmental changes is well known, having been studied by many writers, including the author. Especially significant for our present purposes are the large parental correlations, both positive and negative, which may be produced within a clone by changing environment (1914). In order to discover if there were any *prima facie* justification for suspecting the operation of changing environment (food, temperature, etc.) in producing the parental correlation just described, the six months during which the hybrid clone was under observation were divided into nine periods of three weeks each (roughly the period covered by one parthenogenetic cycle), and the mean ratios for all females born in each period was determined, with the following result:

Period	Number of females born in this period	Mean $\frac{B}{A}$ ratio
1. April 19—May 9	29	1·995
2. May 10—May 30	79	1·991
3. May 31—June 20	61	2·063
4. June 21—July 11	16	2·050
5. July 12—August 1	17	1·922
6. August 2—August 22 ...	21	1·879
7. August 23—September 12 ...	10	1·772
8. September 13—October 3 ...	28	1·935
9. October 3—October 24 ...	36	1·920

The fact that the means for the last four 3-week periods are all lower than those of any of the first five, obviously tends to produce a

[1] Two females of the clone were omitted from this, and all other calculations, owing to abdominal deformity.

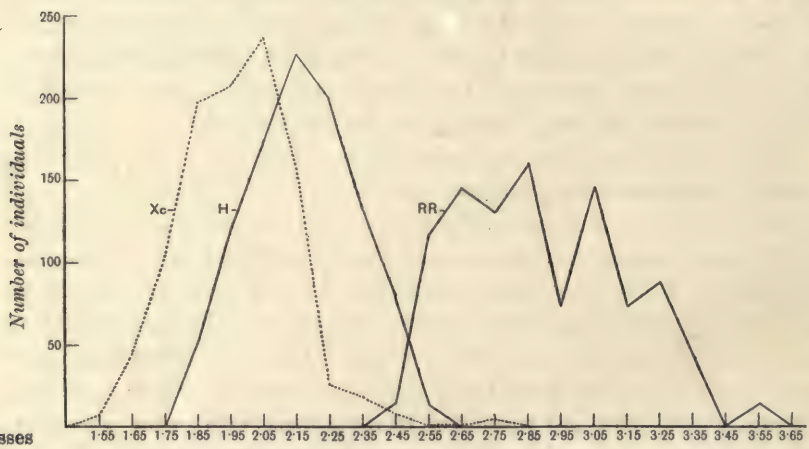
positive parental correlation, since the majority of mothers born in the first 15 weeks are correlated with offspring born in the same period, and correspondingly for the last 12 weeks. It is evident therefore that in order to reduce the effect of environmental modification, and allow true genetic differences (if such exist) to manifest themselves, a correction must be applied to the original measurements which will counteract the modifications produced by environment. The correlation table was therefore prepared in a new way. The difference between each individual ratio and the mean ratio of all individuals born within five days before and after it, was found, and the correlation table was reconstructed with these values. In this way the effects of environment are more or less smoothed over, since each individual, instead of being compared with the mean for the total population is only contrasted with those individuals born at about the same time, and therefore subjected to more similar conditions. The method does not provide an entirely satisfactory correction factor, since 10 days is in some cases not long enough to include a satisfactorily large number of individuals, and yet is long enough to allow of a certain amount of change in the environment.

The parental correlation as found in this way is $\cdot 115 \pm \cdot 040$, somewhat less than three times its probable error.

Moreover, if we turn our attention to the few individuals with the highest ratios (and since the mean for the hybrid clone is less than that for either of the parental clones, these are the only cases which need be considered as evidence of segregation), we find that the highest hybrid ratios were one of 2·75 and two of 2·45 (cf. Fig. 3). Unfortunately no offspring were obtained from these three individuals. There were four mothers of ratio 2·35, and these had 14 daughters with mean ratio 2·01. There were also four mothers with ratio 2·25, and these gave 11 offspring also with a mean of 2·01. These means are slightly above the means for the hybrid clone, and hence are responsible for part of the positive correlation coefficient, but they are below the mean for the clone *H*, and much lower than the lowest ratio found in the *RR* clone. If we examine, not only the daughters, but the total descendants of these eight mothers, we find that the four with ratio 2·35 gave a total of 27 female descendants with a mean ratio of 1·96, and the four parents with ratio 2·25 produced 42 female descendants with a mean ratio of 1·99. As the mean of the whole hybrid clone is 1·97, it is plain that the high ratios of these females, though it chanced to re-appear to a slight degree in their daughters, was not maintained in their remoter female descendants. These eight mothers also produced together a total of 48 male offspring,

and their mean ratio was 10·4, practically the same as that of the hybrid clone (10·1).

A confirmation of the absence of segregation within the females of the hybrid clone is the absence of bi- or multi-modality in the distribution of the ratio values. Fig. 3 shows the distribution of each of the two parental clones, and of generations 3—10 of the hybrid clone. The latter shows no sign of multi-modality, though such would almost certainly manifest itself in the very early generations from the original hybrid if segregation were taking place. Moreover, it is noticeable that the extremes of the curves of all three clones are stretched out asymmetrically towards the right (high ratio), and doubtless in the hybrid clone this skewness is due to the same causes as in the parental clones—i.e. not due to segregation but to non-genetic causes.



Classes	1·55	1·65	1·75	1·85	1·95	2·05	2·15	2·25	2·35	2·45	2·55	2·65	2·75	2·85	2·95	3·05	3·15	3·25	3·35	3·45	3·55	3·65	Totals
Xc	2	12	28	54	57	65	43	7	5	2	0	0	1	—	—	—	—	—	—	—	—	—	276
H	—	—	—	4	9	13	17	15	10	6	1	—	—	—	—	—	—	—	—	—	—	—	75
RR	—	—	—	—	—	—	—	—	—	1	8	10	9	11	5	10	5	6	3	0	1	—	69

Means			Standard Deviations		
	Xc	1·97		*Xc*	·169
,,	*H*	2·17	,, ,,	*H*	·166
,,	*RR*	2·89	,, ,,	*RR*	·254

Fig. 3. Distribution of the $\frac{B}{A}$ ratio in females of clones *Xc*, *H*, and *RR*. For convenience of comparison, the curves are all constructed for 1000 individuals. The actual numbers of individuals which provided the data are shown beneath the curves.

Finally, the fact which was established above, that segregation does not manifest itself among the hybrid male population implies that this was absent also among the females which produced the males.

The Genetics of another hybrid clone.

In addition to the hybrid clone *Xc* just described, another (*Xf*) was obtained from the reciprocal cross *D. pulex* × *D. obtusa*. This clone had all the general characteristics of the clone *Xc*, but the partial sterility of the females was even more marked, so that indeed I failed to maintain the clone for more than four generations, and during that time obtained very few specimens.

The mean $\frac{B}{A}$ ratio for the eleven females measured was 2·24. Only four males were obtained, and of these only three (all sterile) survived for measurement. Their mean $\frac{L}{B}$ ratio was 11·23.

DISCUSSION.

The experiments just described form a confirmation of my former experiments on inheritance in parthenogenesis (1914). The most complete of these employed another Cladoceran, closely related to *Daphnia*, namely *Simocephalus exspinosus*. A large number of ephippia, each of which in *Simocephalus* contains a single fertilized egg, were collected and hatched, and a population was constructed of 57 clones, each descended from one of these fertilized eggs. This was termed the *polyclonal* population, and was bred for five generations. The parental correlation coefficients for the factors size at birth and at maturity, were found for each of the four pairs of generations (1 and 2, 2 and 3, 3 and 4, 4 and 5). The three possible grandparental correlations, two great-grandparental and one great-great-grandparental correlations were also found. All the correlation coefficients, for all degrees of ancestry, were sensibly equal (Table VIII).

TABLE VIII.

Mean correlation coefficients for the polyclonal population of S. exspinosus.

	Size at Birth	Size at Maturity
Parental	·410	·395
Grandparental	·392	·413
Great-grandparental	·408	·505
Great-great-grandparental ...	·430	—

The conclusions to be drawn from these figures are that the original 57 females which gave rise to the 57 clones belonged to a number of different genotypes which reproduced their characters generation after generation, so far as they were not obscured by environmental modifications (if these had been absent the correlation coefficient must have been

unity for each degree of ancestry). Genetic diversity, although it existed between different clones, did not arise between parent and offspring, as shown by the fact that offspring do not resemble their parents more closely than they do their great-great-grandparents. The characters of the individuals of each generation are determined, apart from temporary environmental modifications, solely by the genotype of their clone, and not in any way by the individual characters of their parents or of any other particular ancestor.

This conclusion was completely confirmed by a parallel experiment in which the population was composed of 69 lines descended from the same number of females, not however derived from separate fertilized eggs, but all descended parthenogenetically from a single known ancestor. The constants calculated from this *monoclonal* population are reproduced in Table IX for comparison with Table VIII. It will be seen that the coefficients are some negative and some positive, but also sensibly zero.

TABLE IX.

Mean correlation coefficients for the monoclonal population of S. exspinosus.

	Size at Birth	Size at Maturity
Parental	−·025	−·007
Grandparental	·014	−·062
Great-grandparental	−·098	−·113
Great-great-grandparental ...	·066	—

Thus no inheritable differences existed or arose among the members of the monoclonal population.

As pointed out in the paper from which these two tables have been extracted, the results imply absence of Mendelian segregation in the parthenogenetic reproduction of the Cladoceran, *S. exspinosus*. The experiments described in the present paper contain direct confirmatory evidence on this point.

What for convenience may be termed the genotype theory of heredity rests on the fundamental hypotheses that (*a*) the hereditary material consists of relatively stable discrete units located in the chromosomes, and (*b*) that genetic diversity between parent and offspring is due to one of two causes: either to mutation, or, much more frequently, to the segregation of homologous chromosomes in meiosis and their recombinations at syngamy. The latter part of this dual thesis is capable of direct experimental test. If genetic diversity (except for mutation) is due to the processes of meiosis, it should not arise in any form of reproduction in which meiosis does not occur. This test has been applied, with success

to the theory, over and over again in regard to asexual propagation in plants. The theory has now successfully stood the test in the case of parthenogenetic reproduction, without meiosis, in an animal.

There is of course no reason to suppose that parthenogenetic reproduction, as such, must exclude segregation. It is the absence of meiosis which is the crucial point, and we know that in *Daphnia* and *Simocephalus*, parthenogenetic eggs, whether ♂- or ♀-producing, are matured without reduction of chromosomes (Kuhn, Chambers, Taylor). It is plain that in the parthenogenetic production of haploid eggs from diploid mothers segregation might be expected; and indeed it appears to have taken place in the stick insects investigated by Fryer.

In the light of our general knowledge of genetics, and in particular of the experiments just described in Cladocera, we must postpone our interpretation of the segregation with crossing over, which Nabours found in the parthenogenetic reproduction of *Apottetix*, until the cytology of its oogenesis has been worked out.

The sexual mode of reproduction is indeed so generally associated with meiosis of the type found in the Metazoa and Metaphyta, and the asexual mode with lack of meiosis, that the words sexual and asexual are often used as if tacitly implying such association. The assumption involved by this use of the word must however be kept constantly in mind. Thus, interesting though they are in themselves, and promising of still more interest when properly correlated with cytological observations, the recent experiments on the production by selection of genetic diversity among the asexually produced offspring of certain Protozoa must not, until further knowledge is available, be compared too closely with the production of a similar diversity among Metazoa, in which all the chromatin is concentrated during cell division into chromosomes which are accurately distributed by the mitotic processes.

It must however be borne in mind that exceptions occur to every rule. Although segregation does not normally occur in the absence of the meiotic processes which precede gametogenesis, it is only to be expected that among the many abnormalities that are known to occur in ordinary somatic mitoses, some may result in irregular distribution of the chromosomes leading to segregation of the contained factors. Cases of somatic segregation have indeed often been described in plants, though in the majority of these there seems to be no valid reason for ascribing the phenomena to segregation rather than to mutation.

SUMMARY.

A hybrid clone, derived from the cross *Daphnia obtusa* × *D. pulex* was bred for ten generations. Throughout this period the hybrid clone was characterized by excessive production of males and sexual eggs, complete sterility of the males, partial sterility of the parthenogenetic eggs, and probably normal fertility of the sexual eggs.

The ten parthenogenetic generations of this clone furnished no evidence of the occurrence of segregation.

This experiment is complementary to my former experiments on inheritance in parthenogenesis in the Cladoceran *Simocephalus*, and indicate that during the parthenogenetic reproduction of these forms, which is not accompanied by reduction of chromosomes, Mendelian segregation does not take place, nor does genetic diversity between parent and offspring normally arise.

REFERENCES TO LITERATURE.

AGAR, W. E. *Phil. Trans. Roy. Soc. London*, Series B, Vol. CCIII. 1913.
—— *Phil. Trans. Roy. Soc. London*, Series B, Vol. CCV. 1914.
CHAMBERS, R. *Biological Bulletin*, Vol. XXV. 1913.
DETLEFSEN, J. A. *Carnegie Institute Publication*, No. 205, 1914.
FRYER, J. C. F. *Journal of Genetics*, Vol. III. 1913.
KUHN, A. *Archiv für Zellforschung*, Vol. I. 1908.
NABOURS, R. K. *Journal of Genetics*, Vol. VII. 1917.
TAYLOR, M. *Zoologischer Anzeiger*, Vol. XLV. 1915.

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